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# The Journal of Laboratory and Clinical Medicine

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## ORIGINAL ARTICLES

### POISONOUS PROTEINS*


BY VICTOR C. VAUGHAN, M. D., ANN ARBOR, MICH.


#### INTRODUCTION.

*ORIGIN of Proteins.*—In nature all proteins are the products of life and each kind of living molecule elaborates and contains its own specific protein. Some forms of life are capable of constructing their proteins out of inorganic matter, while others can utilize only that which has been built up by other cells into protein material. Plants take the ammonia, nitrates and nitrites of the air, soil and water and by synthetical processes convert these into the proteins found in their tissues. In this process there are two stages. In the first the inorganic nitrogen is synthesized into amino acids and in the second these are combined to form proteins. The higher animals cannot synthesize inorganic nitrogen into amino acids. This is done for them by plants and to some extent by bacteria in conjunction with plants. By the symbiotic action of certain bacteria and plants even the free nitrogen of the air is drawn upon in the construction of vegetable proteins. So far as protein metabolism is concerned the vegetable world is the synthetical or constructive laboratory while the animal is the analytical or destructive machine. The plant takes the smallest parts and builds them up into highly complex bodies, while the animal takes the complex and splits them into pieces to be reconstructed in its own body. In a general way the above statement is true, but there are synthetical processes going on normally in the animal body and it is demonstrable that simple proteins may be built into more complex molecules in the animal body. Moreover, it is certainly true that in man with perfect digestion practically all the nitrogen of the food is absorbed in the form of amino acids. The animal as well as the plant is a synthetical laboratory, but the new material used by the former is the finished product of the latter, which is unravelled and then woven into a new pattern which is different in each species of animal.

*The Herter Lectures for 1916 given in the University and Bellevue Medical School, New York.

There are as many kinds of proteins as there are kinds of living matter. Chemically proteins are polymers of amino acids. The amino acids demonstrated in proteins are only about eighteen in number, but with these put together in an almost infinite variety of ways, we get an unlimited variety of products, just as with only twenty-six letters in the alphabet there is no end to the making of words. The simplest proteins consist wholly of amino acids. These combine with inorganic salts, lime, phosphorus, iron, etc., and with carbohydrates to form the compound proteins.

All living things not only contain protein, but this is their essential constituent. The living protein molecule is in a labile or active state, capable of trading in energy, absorbing and eliminating; never in a condition of equilibrium. Dead protein is in a state of rest; it is a stabile molecule and remains in equilibrium.

### BACTERIAL PROTEINS.

*Material.*—Fifteen years ago, after various attempts to secure bacterial cellular protein in large amount, I succeeded with the tanks for massive cultures which have been described elsewhere. The growths thus obtained are freed from extraneous matter by washing with dilute alcohol and then by thorough successive extractions with absolute alcohol and ether. The cellular substance is ground first in porcelain and then in agate mortars, and passed through fine meshed sieves. Whatever the bacterium employed the product is a fine white powder. The dilute alcohol removes the extraneous matter mechanically held by the growths and the long continued extractions with alcohol and ether remove coloring matters, fats, waxes, and other less known bodies. I have never made a close study of these extractives. These bacterial powders when examined microscopically show the individual cells plainly, especially when properly stained. Even the chromogenic bacteria come through as white powders, all the color being removed by the alcohol and ether. The freedom of this cellular material from extraneous matter is best appreciated from the fact that when one gram of it is incinerated there is no trace of chloride in the ash. With chloride of sodium in the culture medium and considering the ease with which traces of chloride are detected, this indicates a surprising degree of purity in the material. The significance of the absence of chloride will be discussed later. The cellular protein of many pathogenic and nonpathogenic bacteria has been obtained by growth in the tanks. I may say that I have never dared to grow anthrax bacilli in these massive cultures and have contented myself so far as this organism is concerned with the less abundant growths in Roux flasks. For obtaining abundant growths of the tubercle bacillus the tanks are less suitable than glycerine beef-tea cultures.

*Chemistry.*—It has been generally assumed that bacteria are unicellular plants. This assumption rests, so far as I can learn, upon early statements, such as that of Pollender, that anthrax bacilli are not affected by strong alkali and this has been interpreted as meaning that they consist largely of cellulose. It is true that certain investigators have claimed to demonstrate even large amounts of cellulose in bacteria. Hammerschlag on wholly inadequate evidence estimated the per cent of cellulose in the tubercle bacillus as high as 28.1. DeSchweinitz and Dorset reduce this amount to 6.95 per cent, but hardly accept this figure

themselves since they conclude that cellulose is probably present in small amount in the tubercle bacillus, and not present in the bacillus of glanders. These and other investigators, who have reported the presence of cellulose in bacterial cellular substances, have not properly distinguished between cellulose and other carbohydrates. Vincenzi employing proper tests failed to find cellulose in the cellular substance of bacillus subtillis, but did find a nitrogenous carbohydrate. In our work, Wheeler made special search for cellulose in sarcina lutea. Twenty grams of cell substance was autoclaved with 25 parts (500 c.c.) of ten per cent potassium hydroxide at 120°, first for thirty minutes and then for an hour. There remained a considerable residue which gave none of the protein reactions, did not reduce Fehling's solution even after prolonged boiling with dilute hydrochloric acid, but did respond to the carbohydrate test with alpha-naphthol. Cellulose could not be detected by any known test. Schweitzer's reagent failed to dissolve it and it gave no color with iodine even after treatment with sulphuric acid. A portion was dried and heated with soda lime when it evolved a gas which turned red litmus paper blue, thus indicating the presence of nitrogen which had been reduced to ammonia. The odor of burning feathers also indicated the presence of nitrogen. From these results we conclude that there is no evidence of the presence of cellulose in bacterial cellular substance. Leach made search for cellulose in the cells of the colon bacillus with like negative results. Like Vincenzi we did find a nitrogenous carbohydrate. This is chitin or some chitin-like substance. The presence of chitin in bacterial cell substance has been reported by Ivanoff, Emmerling, Helbin, Bulloch, and others.

My students and I have found two carbohydrates in bacterial cellular substance. One, referred to above, is combined with nitrogen, is not soluble in strong alkali, and does not reduce Fehling's solution even after prolonged boiling with dilute mineral acids. The second carbohydrate is combined with phosphorus, is soluble in alkali, and does reduce Fehling's solution after being boiled with dilute mineral acid. In the unbroken molecule this carbohydrate undoubtedly is contained within the nuclein group. If the presence of cellulose be essential to plant tissue, bacteria certainly are not forms of plant life.

There is no controversy concerning the presence of nuclein in bacterial cellular substance as the xanthine bases have been demonstrated among the disruption products both in my own laboratory and elsewhere. The literature on this subject is too extensive to permit me to go into it exhaustively and I will content myself with a few references. Klebs obtained from the tubercle bacillus a nuclein containing 8-9 per cent of phosphorus. From the same organism Ruppel separated a nuclein containing 9.42 per cent of phosphorus which he designated tuberculinic acid. Levin obtained from the tubercle bacillus proteins, nuclein, and crystals which he considered a mixture of thymil and uracil, also cystosin. Lustig and Galeotti obtained a nucleoprotein from the pest bacillus. In our work the presence of nuclein bodies was plainly in evidence. Leach obtained from the colon cell substance a body containing 7.33 per cent of phosphorus and both Leach and Wheeler secured evidence of the presence of xanthine bases.

Bacterial cellular substance responds to all the protein reactions. Proteins are detached from the substance by both alkalis and acids, but the properties of the bodies thus obtained indicate that they are split products obtained by the

cleavage of more complex molecules, and do not exist free in the cellular substance. Dilute mineral acid splits off the nitrogenous carbohydrate and when this extraction is carried on at high temperature much of the second carbohydrate is converted into a reducing substance. The acid extracts when dropped into a large volume of alcohol give a precipitate which after purification by resolution in water and reprecipitation with alcohol yields more than seven per cent of phosphorus. The line of cleavage through the large molecules in the cellular substance followed by acid action seems to be definite and the same products are obtained with one per cent and 0.1 per cent sulphuric acid. More concentrated acids after prolonged heating break deeper into the molecular structure and cleave the biuret bodies with the liberation of amino acids. Wheeler and Leach have made special studies of the action of mineral acids on bacterial cellular substance. Ten per cent solutions of potassium hydroxide at 120° extract from bacterial cell substance everything except the chitin-like body which consists of a carbohydrate combined with nitrogen.

We have demonstrated the presence in bacterial cellular substance of both mono and diamino acids and have shown that the percentage of these varies with the microorganism. We have found the percentage of nitrogen to vary from 5.964 in subtilis to 11.765 in violaceus.

I began this work with the expectation of finding the bacterial cell substance composed of relatively simple bodies. I have been compelled to come to the opposite conclusion. The cellular substance of bacteria contains highly complex molecules. We have demonstrated the presence of the following groups among the split products: (a) a chitin-like body consisting of a carbohydrate combined with nitrogen. It seems reasonable to infer that this exists in the cellular substance as a glyco-protein. (b) A carbohydrate group combined with phosphorus from which it is not easily detached. This group reduces copper after prolonged boiling with dilute mineral acid. The amounts as determined by the reduction of Fehling's solution and calculated as xylose are large, but we are not sure that the reducing substance is all carbohydrate. Indeed, it might be better to speak of both of these groups as those responding to the Alpha-naphthol test rather than as carbohydrates and to distinguish between them as nonreducing and reducing bodies. However, it seems clear that the one now under consideration is a subgroup in the nucleinic acid constituent of the cell substance. (c) The presence of nucleinic acid is beyond doubt, as is shown by the high phosphorus content of some of the split products and by the demonstration of the xanthin bases. (d) That one or more protein groups exist in the cell substance. If all these groups exist in the same molecule the cell substance must contain a highly complex molecule which would be best designated as a glyco-nucleo-protein. The fact that these bodies are removed only by agents capable of causing molecular disruption inclines me to the belief that the molecules which make the cell substance are highly complex. It may be said that this is an assumption and without adequate proof. On the other hand, such a statement as that made by Doerr, that bacterial proteins are of simple molecular structure is wholly without evidence. Because bacteria are simple morphologically is no proof that they are made up of simple proteins. This

certainly is not true even if it should prove that I have over-estimated the size of these protein molecules.

Tamura working in Kossel's laboratory has made a contribution to the chemistry of bacteria. He used cellular material obtained from the bacillus tuberculosis and mykobacterium lacticola perrugrosum. Both of these were grown in glycerine-broth cultures for five weeks, then collected on filters and washed with ether and alcohol. Tamura states that all the fats and waxes cannot be removed in this way and he resorted to the following method: After partial extraction with ether and alcohol the bacterial cells were rubbed up in a mortar with two parts of sulphuric acid and one of water and from this mass extraction with ether and alcohol was continued. From these extracts Tamura obtained along with the well known fatty acids diamino monophosphatide, a substance which has been previously found in egg-yolk, muscle and brain. Tamura thinks that this body has been mistaken for lecithin by other investigators working with ether and alcohol extracts of bacteria. In these extracts Tamura has furthermore detected a higher alcohol, which he names "mykol" and to which the "acid fast" properties of these bacteria are due. The statement that the fats and waxes including the phosphatide and "mykol" cannot be removed from the tubercle bacillus with alcohol and ether without previous disruption with strong acid is erroneous. In our work, first published in 1908, we showed that prolonged extraction of tubercle cell protein with alcohol and ether removed from the cells the substance to which the "acid-fast" property is due. We extracted first with alcohol in Soxhlets for four days and then with ether for three days. There is therefore no ground for the assumption by Tamura that either the phosphatide or the "mykol" are in chemical combination with the proteins of the cellular substance. It is much more reasonable to regard them as substances either on their way to assimilation into the cell molecules or as excretory products. Tamura says: "My investigations show that the presence of the diaminophosphatide is not confined to the higher organisms." This is another assumption that the molecular structure of bacterial cells is simple and is wholly without justification. It is additional support of my contention that chemically the bacterial cell is highly complex and should not be regarded as a primitive form of life. Tamura's work on the cellular proteins strongly supports my claim that these are highly complex in chemical structure. He states that he was not able to extract protein from the cell substance with water, salt solution, or one per cent sulphuric acid, and that even with alkali a portion of the cell substance remained undissolved. Surely this would not be true if the cell substance consisted of simple proteins. He also obtained the nuclein bases, diamino and monoamino-acids. Of the last mentioned his list for the tubercle bacillus contains one (prolin) not found in ours, while ours contains two (glutamic acid and leucin) not found in his. Neither found glycocoll in the tubercle bacillus while we found it in the colon bacillus.

Tamura concludes, as we had done some years before, that bacterial cellular substance contains two carbohydrate groups, but the one which we have designated as chitin-like body, he classifies as a hemicellulose. This name was proposed by Schultze, after an investigation of various cell membranes, to include a group of bodies, "which are wholly soluble on being heated with dilute

alkalis. In the cold five per cent sodium hydroxide dissolves them somewhat more slowly." And yet Tamura classes as a hemicellulose a body which remains in the residue after repeated extraction with one per cent sodium hydroxide; besides he did not test this residue for nitrogen but seems to have assumed that this element was not present since the body did not respond to the protein reactions. In our tests this body remained in the residue after heating for one hour at 120° with ten per cent potassium hydroxide, and did not give the protein reactions, but did contain nitrogen. We know of no nitrogenous carbohydrates except the chitins.

Tamura reports a negative test for sulphur in bacterial proteins, but this is due to faulty technic, since Wheeler has shown that the sulphur is masked and does not respond to the ordinary tests, but its presence is disclosed when a portion of the substance is fused with metallic sodium, dissolved in water and treated with a freshly prepared solution of sodium nitroprusiate; a violet color indicating the presence of sulphur.

While the "acid fast" and "Gram positive" properties of certain bacteria depend upon lipoids which are extracted from the cells by alcohol and ether, the cells, after exhaustive extraction with these solvents, take the analin dyes quite as well or even better than before. For instance the extracted tubercle bacillus stains just as well, or even better, than before extraction with alcohol and ether, but now the stain is easily removed by dilute acid. This behavior of bacterial cellular substance towards basic analin dyes quite naturally suggests that the former consists largely of nuclear material. In my opinion this is strengthened by the studies of the cellular substance which I have outlined. Additional evidence in the same direction is not wanting. When sporogenous bacteria form spores or pass into the resting stage the essential part of the bacterial cell is contained in the spores and all spores and reproductive cells consist in part at least of nuclear material. Certain bacteria which do not form spores pass into a granular state in which potential life continues for a long time. For instance the bacillus of glanders, though an asporogenous organism, may retain viability for a long time. Wladmiroff states that he found these organisms in glycerine-bouillon tubes, with the ordinary cotton plug capable of growth after standing four years. The same is true of the plague bacillus. This phenomenon is explainable only on the assumption that these bacilli contain nuclein. However, assumption is no longer necessary since nuclein, nucleinic acid and their derivatives have been found in all bacterial cells submitted to chemical study. I am strongly of the opinion that the bacterial cellular substance as I have prepared and studied it, freed from the extractives soluble in water, salt solution, alcohol and ether, is practically all nuclear material.

Some years ago A. B. Macallum by microchemical methods showed that nuclei are free from chlorine. His statement is as follows: "Intercellular material and structures, including the so-called cement substance of von Rechlinghausen are rich in chlorides but normal nuclei of animal and vegetable cells are absolutely free from them." When my coworkers found no chlorine in the ash of our cellular substance I thought that this must be due to careless work. I could not believe that with the chlorides, especially sodium chloride, as abundantly distributed as they are, they could not be wholly wanting in this material,

but repeated examinations confirmed the first finding. The finding of chlorine would not have been a conclusive evidence that the material is not wholly nuclear, but the failure to find any trace of this element I regard as most convincing evidence that it is wholly nuclear. Furthermore, Macallum found that the phosphorus and iron in nuclear material are masked, that is, they cannot be detected without more or less marked disruption of the molecule. This is true of our cellular substance. I may recall the further fact that the sulphur is so masked that even in the laboratory of so eminent a chemist as Kossel it was not detected in bacterial cellular substance. It is well known that in proteins sulphur exists in two forms, one being readily split off with dilute alkali forming a sulphide, while the other is obtained only when the disruption of the protein molecule is carried much further. The former is wanting and the latter present in our cellular substance. I do not suppose that all nuclear material has the same elemental constituents, indeed, it is not supposable that this is true, but the above facts seem worthy of consideration.

The laborious and valuable researches of Macallum have shown that non-nucleated organisms, such as cyanophycea, beggiota and yeast cells, contain nuclein, and this is probably true of every cell which is capable of reproduction. We are no longer quite willing to accept the dictum of Schultze, Hertwig and others that every cell must contain a morphologically recognizable part, known as a nucleus. We may insist upon the presence of nuclear matter, but not of nuclei. Some morphologists have seen the necessity of altering our conception of a cell. Bourne has proposed that Schultze's definition be changed to read: "A cell is a corpuscle of protoplasm which contains a specialized element, nuclein."

It should be understood that the cellular substance which I have been discussing is not identical with that which exists in the living multiple bacteria. The latter consists of the former with the addition of all the extractives which I have removed by the solvents, such as water, dilute alcohol, absolute alcohol and ether. The living bacillus has been stripped of all its surrounding food supplies, its accumulated excretory products and its storehouse of fats, waxes, etc. I have a strong suspicion that in some of our bacterial reactions, notably with precipitins and agglutinins, these extractives are concerned, while the cellular constituents have no direct part. The active constituents of the culture, the agglutinable substance, is not, in my opinion, an essential constituent of the bacterial cells, but consists of one or more proteins closely associated with the bacterial cells. It may be a protein already split off from the surrounding pabulum preparatory to absorption and assimilation, or it may be an excretory product. My reasons may be stated as follows: (1) Agglutination does not destroy the viability or virulence of bacteria; therefore, the reaction does not disrupt the living bacterial cell. (2) Thoroughly washed typhoid bacilli are not agglutinable. (3) When typhoid bacilli are thoroughly shaken in salt solution so as to remove their flagellæ and the bacilli are deposited in a centrifuge, the emulsion of flagellæ is agglutinable. (4) Neufeld has shown that when cholera bacilli are thoroughly cleansed by being shaken with one per cent alkali, which does not destroy them and only washes away adherent matter, they are not inagglutinable but produce no agglutinin when injected into animals.

Agglutination and precipitation are closely related phenomena. When a bacterial culture is filtered some of the proteins about the cells pass into solution and constitute the precipitogen while some of the same class of near-cell proteins remain adherent to the cells and constitute the agglutinable substance or the agglutinogen.

I find that the bacterial cellular substances on standing undergo autolytic cleavage. We are just now examining a bottle of colon cellular substance which was prepared ten years ago. It was only air dried and contains a small amount of moisture. When freshly prepared water or salt solution extracted no protein, now one-third the nitrogen passes into solution when the substance is treated with these solvents.

*Poisonous Action.*—We have found all the bacterial cellular proteins poisonous. Our earlier work was done with the colon cell substance. Since all of these bodies are insoluble in water or salt-solution it has been necessary to administer them in suspension. Early studies demonstrated the following facts: (1) The poison is contained within the bacterial cell and does not under ordinary conditions diffuse into the culture medium.* It is true that old cultures may contain soluble poisons, but these result from autolysis. (2) The poison is not extracted from the cellular substance by water, saline solution, alcohol or ether, either at ordinary temperature or at the boiling point. (3) Heating, even to 140° in the autoclave does not destroy the poison. (4) Dilute (0.5 per cent) solutions of caustic alkali disrupt the cellular substance slowly and imperfectly. Stronger (2 per cent) solutions break up the cell substance and render the poisonous fraction soluble. (5) Boiling with dilute mineral acid (to 1 per cent) has but little effect.

At first we were much puzzled by the fact that smaller doses killed while larger ones failed to do so. This was observed when we were administering the substance by intraperitoneal injection. Then we found that the more finely the substance was ground the smaller was the fatal dose. When the substance was only coarsely ground in a porcelain mortar and suspended in water it did not kill guinea-pigs on intraperitoneal injection in doses less than 1 to 40,000 parts body weight. When the same powder was more finely ground in an agate mortar it killed 15 out of 16 animals at 1 to 75,000; 9 out of 28 at 1 to 100,000; 5 out of 8 at 1 to 200,000; 4 out of 34 at 1 to 2,000,000 body weight. We observed that when heavy suspensions were used lumps of the substance remained undissolved in the peritoneal cavity after death or recovery. In these observations we found the solution of our puzzle. The poisonous action of the cellular substance is in proportion to the extent to which and the rapidity with which, it is split up by the secretions of the body cells and this cleavage is determined by the relative surface exposure of the substance to the action of the cleavage agents. I dare say that the difference in susceptibility as shown among the individual animals is due to the abundance and effectiveness of the secretions elaborated by the body cells.

As has been said, we found the cellular proteins of all the bacteria studied more or less harmful to animals when introduced parenterally. The size of the

*It is understood that we are speaking of cellular poisons and not of bacterial toxins.

dose necessary to produce a fatal result varies greatly with the source of the protein. The cellular substance of bacteria to which in its living state an animal is highly susceptible does not kill that animal at all or does so only after large doses. We have injected into the abdominal cavities of guinea-pigs the cellular proteins of the tubercle bacillus in quantities of from five to two hundred mg. without causing death in a single instance, while on the other hand a fraction of a mg. of the protein from bacillus prodigiosus kills. To kill a guinea-pig one part of the cellular substance of the anthrax bacillus to 1,700 parts of body weight is necessary, while with the colon substance one part to 75,000 kills all animals provided the material is finely ground. In general it may be said that the more highly susceptible a given animal is to infection with a given bacterium the more difficult it is to kill that animal with the cellular protein of that bacterium. On the other hand, the more highly immune a given animal to infection with a given bacterium the more readily does that animal succumb to injections of the cellular proteins of that bacterium. At first sight these statements seem wholly irrational, but when we study them we find that they are not only reasonable but in accord with what might have been reasonably predicted beforehand. The guinea-pig is highly susceptible to infection with the tubercle bacillus because the secretions of its body cells have no destructive action on that organism. This together with the fact that the bacillus tuberculosis can feed upon certain proteins in the guinea-pig's body are the essential factors in the susceptibility. The infecting bacillus finds an abundance of suitable food and meets with no resistance. On the other hand the guinea-pig is highly immune to infection with the bacillus prodigiosus because the animal's body cells supply secretions which are immediately destructive to this organism and the first of these bacilli finding their way into the animal's body meet with immediate and complete annihilation. But when the prodigiosus is grown in vitro and a sufficient amount of its cellular substance, dead or alive, is thrown into the abdominal cavity the same agency which has given the animal immunity to infection now causes it to fall a victim to the protein poison. These facts are of practical as well as scientific interest because they undoubtedly form the basis of the frequently reported and well attested observations of some of the great clinicians of the past that the case mortality in certain infections, most notably in typhus fever, is much higher in the better nourished than in the less robust.

As I have indicated the cellular proteins when introduced parenterally into animals are not wholly harmless even when they do not kill. When the cellular substance of the bacillus tuberculosis is injected into the abdominal cavity of a guinea-pig it has no recognizable effect so far as the behavior or external condition of the animal shows. The dead bacilli are taken up in the folds of omentum and develop local tubercles. When the cellular substance of the colon bacillus is injected, a peritonitis results. In short, the lesions which follow infections result also from the injection of the dead cellular substance. I conclude from this that the lesions of the infections are not due to the activity of the living bacilli, but result from reaction between the bacterial proteins and the body cells.

*Split Products.*—In 1903, Wheeler and I found that the bacterial cellular proteins could be split into poisonous and nonpoisonous parts and later we showed that all true proteins can be broken up in the same way. This work has been

confirmed by many investigators. There are several ways in which this cleavage can be secured, but the most satisfactory is the one which we first employed. The dried protein, after exhaustive extraction with alcohol and ether, is repeatedly heated at 78° with a two per cent solution of sodium hydroxide in absolute alcohol. When this is done the poisonous fraction goes into solution while the nonpoisonous part remains undissolved and is removed by filtration. This is evidently a true cleavage and not a mere disintegration. The nonpoisonous portion contains all the carbohydrate and phosphorus of the original complex molecule.

*The Protein Poison.*—Since this body has been obtained from all true proteins, bacterial, vegetable and animal, so far examined, we have called it "the crude soluble poison;" "crude" because it is undoubtedly a mixture of chemical bodies and "soluble" in contradistinction to the bacterial cellular proteins from which it was first prepared. Aqueous solutions are somewhat opalescent, and may be quite turbid. Filtration through hard paper generally gives a clear filtrate but with some preparations we have found filtration through porcelain necessary to secure a perfectly clear solution. All the crude soluble poisons that we have obtained give the biuret and Millon tests. None give the Molisch test, thus showing the absence of carbohydrate. Some give the Adamkiewicz and Liebermann tests while others do not. This test is believed to be due to the presence of tryptophane. The fact that the poisons from certain proteins do not respond to these tests indicates that Doerr's assumption that the poisonous action is due to the presence of this group is without support. The poison gives the Millon test most strikingly and in high dilution. This test is believed to indicate the presence of tyrosine and it is interesting to note that gelatine which contains no tyrosine does not yield the poison. Aqueous solutions are distinctly acid to litmus and this reaction is due to some organic body. Neutralization with alkalis and alkaline earths weaken the action of the poisons. Poisons from some proteins appear to form definite compounds with calcium and magnesium and at least some of the calcium bodies are inert. In the dry state the protein poison forms a brownish powder varying somewhat in shade with the protein from which it is obtained. All preparations have the same marked odor. It is much more freely soluble in absolute alcohol than in water. Whether it should be called a protein or not is a question. Proteins should not be soluble in absolute alcohol. However this substance gives the biuret test and this is generally regarded as the most distinctive test for proteins. Its alcoholic solutions are precipitated by alcoholic solutions of copper, mercury and platinum. By means of these precipitants with subsequent removal of the metal with hydrogen sulphide, we have obtained our most potent preparations. By this method we have obtained a body which kills guinea-pigs of from two hundred to three hundred grams weight in doses of 0.5 mg. given intravenously. The poison is not an alkaloid, although it may be basic in character.

*Action on Animals.*—The comparative effects of the living bacillus, the dead cellular substance and the crude soluble poison on animals was first worked out by V. C. Vaughan, Jr. The organism used was the colon bacillus.

(a) The Living Bacillus.—When a guinea-pig receives a fatal dose of the living colon bacillus intraperitoneally there is a period of from five to twelve hours, varying with the size of the inoculation, during which there are no recog-

nizable symptoms. We regard this as the period of incubation, and it is roughly proportional to the amount of the culture used and to some extent to the virulence of the organism or the rate at which the bacillus multiplies. This work was done with a bacillus, 1 c.c. of a twelve hour or older bouillon culture of which invariably killed within twenty-four hours. When this amount was given no effects became visible for a period of from ten to twelve hours. With larger doses the period of incubation was somewhat shorter, but with the largest doses of the richest cultures there is still a period of incubation. This measures the time necessary for two things to happen. First the bacillus must multiply sufficiently to supply enough poison to visibly affect the animal. Second, this poison must be made effective by being split out of the large molecule of which it is a part. Therefore, while the period of incubation is not accompanied by the development of symptoms which rise to the plane of observation, it is actually a critical period in every infection and the outcome depends upon whether the bacteria are all destroyed before a lethal dose of the poison has been developed by the multiplication of the bacillus and set free or made effective by the secretions of the body cells. It is during this period that natural and acquired immunity either save the day or, for the time at least, fail. In natural infection the number of bacilli introduced is small and in case of full immunity these are all destroyed, there is no multiplication and the amount of poison set free in the destruction of the small number of the invaders is not sufficient to induce symptoms or to develop lesions. This is what happens when the smallpox virus finds its way into the body of one thoroughly immunized by a previous attack of the disease or by successful vaccination. When the immunity is only partial or when the infection is massive or unusually virulent, the virus develops for a time, becomes more or less distributed in certain tissues and its final destruction is accompanied by the development of symptoms, and the reaction between the virus and the body cells leaves more or less marked lesions. When there is no immunity the virus multiplies without hindrance and life is destroyed. There are infections in which the body shows little or no resistance. Some of these run an acute course and destroy life in a few days, while others are more chronic. This seems to depend upon the rate of multiplication in the invading organism. Apparently there is relatively as much difference in the rate of multiplication in bacteria as there is among the higher animals. The "generation period" or the interval between fissions varies among species and strains, and is influenced by external conditions. Virulence is largely determined by rate of multiplication or at least the two correspond. Under favorable conditions the cholera bacillus divides about every half hour. So far as I know no one has determined the "generation period" in the tubercle bacillus, but it is certainly much longer. It follows that cholera is an acute disease, often terminating fatally in a few hours, while tuberculosis extends through months and even years. The guinea-pig shows no resistance to the tubercle bacillus and the organism slowly but steadily grows, develops its characteristic lesions and kills, probably through its autolytic products and without developing any antagonistic action in the body cells. Rodents, especially rats, show but little or no resistance to the plague bacillus, except in those regions where this disease is endemic and there, it is said, this disease even among the rats becomes a chronic infection.

Our intraperitoneal infection of the guinea-pig is comparable with the development of a general peritonitis from a ruptured appendix. The period of incubation is short and while there may be some elevation of temperature, this is not marked or even constant. During the period of incubation, when the bacilli are abundantly multiplying, the behavior of the animal in no way distinguishes it from its untreated fellows, but at the end of this period there is a marked change. The animal no longer eats; its coat becomes rough; its head droops; it sits in one corner of the cage in a stupor; its abdominal walls become rigid and pressure over this region elicits evidence of pain. Now, its temperature begins to fall and this decline is progressive in fatal cases. We have frequently seen the temperature fall from 101° to 94° in from two to four hours and it may reach 85° and even lower before death. A rise in temperature after it begins to fall generally means recovery. Autopsy reveals a general hemorrhagic peritonitis with a large amount of bloody fluid with intact red corpuscles and leucocytes in the peritoneal cavity. The parietal and visceral peritoneum are studded with minute punctiform hemorrhages and there is more abundant hemorrhage in the great omentum. The chemotactic pull of the bacilli has been not only great enough to assemble great numbers of leucocytes, but violent enough to rupture small blood vessels.

(b) The Cellular Proteins.—When a fatal quantity of the cellular protein of the colon bacillus is injected into the peritoneal cavity of a guinea-pig the progress of events is exactly like that following infection with the living organism except that the period of incubation is shortened. There is no longer either opportunity or need for the multiplication of the bacillus. This has taken place in vitro and enough of the protein to kill has been introduced. One of the features that characterize and mark the period of incubation has been withdrawn. It only remains for the body cells by means of their secretions to cleave the bacterial protein and set the poison free. The period of incubation is reduced half or more, then the evidences of poisonous action are exactly the same as in the inoculated animal. The temperature falls at the same rate and autopsy reveals exactly the same lesions. The chemotactic pull of the dead protein has proven just as strong and just as violent as that of the living protein. In fact the pull in both instances is a chemical and not a vital one and the lesions result from a reaction betwen the proteins of the bacterial cells and those of the body cells.

(c) The Soluble Poison.—When a fatal dose of the crude soluble poison is injected into the peritoneal cavity, the effects begin to reveal themselves much sooner. There is now no period of incubation. Both steps, which have characterized this period, are now omitted. The bacillus has been grown and has been cleaved in vitro. The action of the poison begins to manifest itself within a few minutes—from five to twenty—and it appears in three, well marked stages: The first we have designated as that of peripheral irritation. In the guinea-pig it is manifest by the animal scratching itself, generally first on the nose and then over every part of the body which can be reached by its claws. In man an erythematous blush, beginning about the point of injection, spreads over the body and may be followed by an urticarial rash with intense itching. This is not always confined to the cutaneous surface, but may extend to the mucous membrane of the mouth, throat and rectum. The second stage is one of partial paralysis.

The guinea-pig lies on its side, with rapid, shallow and difficult breathing. When urged to move it shows inability to coordinate its movements and partial paralysis is evident, especially in the posterior extremities. In man the breathing becomes distressingly asthmatic. Air-hunger is marked and there is a sense of impending danger. The convulsive stage marks the termination. The convulsions are usually clonic and at first generally involve only the neck muscles, the head being thrown back. The seizures extend over the body, becoming more frequent and violent. During a convulsion, occasionally in an interval, respiration ceases. The heart continues to beat, at first with no acceleration and with perfect regularity. The exact mode differs somewhat in different animals, but is always that of anaphylactic shock. Necropsy shows the same conditions found after death from anaphylactic shock. The peritonitis found after death from inoculation or from the injection of the unbroken cellular protein is wholly wanting.

When a nonfatal dose of the soluble poison is administered, the symptoms are those described above as characterizing the first and second stages. There may be isolated and slight convulsive seizures, but an animal seldom recovers after the convulsions have become general and frequent. With recovery the temperature slowly rises and ultimately returns to normal. Within two hours the animal is apparently quite normal in every respect.

*(To be continued.)*

# THE PHARMACOLOGICAL ACTION OF NITROUS OXIDE*

By D. E. Jackson, Ph.D., M.D., St. Louis, Mo.

IN a previous article[1] I have described a closed method for the administration of nitrous oxide and other anesthetics in conjunction with oxygen. In the present instance I want to discuss certain features of the pharmacological action of nitrous oxide as studied by this method. No morphine, scopolamine or other hypnotic has been used in any of these experiments.

I may refer briefly to an improved form of apparatus which I have used in these experiments (Fig. 1). A rotary pump of less capacity than that used in the device previously described is attached to a very much smaller and more compact frame. The chief object in this has been to simplify the apparatus and to reduce its size and weight. A number of valves, tubes, etc., shown in the illustration have been found by experience to be unnecessary, but in the experimental development of the device they were included as precautionary measures. The apparatus carries only two tanks, one for oxygen and one for nitrous oxide, for experience has shown that since tanks need be renewed only at considerable intervals, and, if the breathing bag be filled moderately full at the moment when either tank becomes exhausted there will be ample time to remove the empty tank and replace it by a new one before a fresh supply of the gas (generally oxygen, of course) is required. Realizing the great value of simplicity and lightness in any form of apparatus intended for constant use, I have spent much time and energy in trying to produce as simple a device as possible. It is perfectly evident that the apparatus here shown is much more complicated than it need be, but for the benefit of others who may be interested in the subject of nitrous oxide anesthesia, I have thought it worth while to include here an illustration of the apparatus with which much of the work discussed below has been carried out. For a description of the general principles on which the device is operated I must refer the reader to the article indicated above. I may state briefly, however, that by means of a motor and a rotary air pump, air or other gaseous or volatile substances (chiefly nitrous oxide and oxygen so far as the present article is concerned) are kept circulating within a closed system of tubes and vessels, and through a breathing bag into and out of which the animal breathes.

The vessels are two in number and consist of glass jars, the one containing sulphuric acid which serves to sterilize, dry and warm the air (or gases) which are washed through the acid, while the other jar contains sodium hydrate solution through which the air or gases, including the exhaled $CO_2$ from the patient are washed. The $CO_2$ is immediately absorbed by the sodium hydrate forming sodium carbonate and water according to the following equation:

$$2\ Na\ OH + CO_2 = Na_2\ CO_3 + H_2O.$$

The sodium carbonate being a soluble salt of course remains in solution (to-

*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.

gether with the $H_2O$ formed) in the jar, the $CO_2$ being thus removed from the air (or nitrous oxide and oxygen) which the animal breathes. During this process the oxygen is consumed (250 to 300 c.c. per minute for an adult man at rest) by the animal or patient. More oxygen is injected into the system from time to time in just such quantities as the animal actually consumes. The nitrous oxide, being a stable gas, is not broken down at all either by the animal or by the acid or sodium hydrate. Consequently there need be but little waste of the

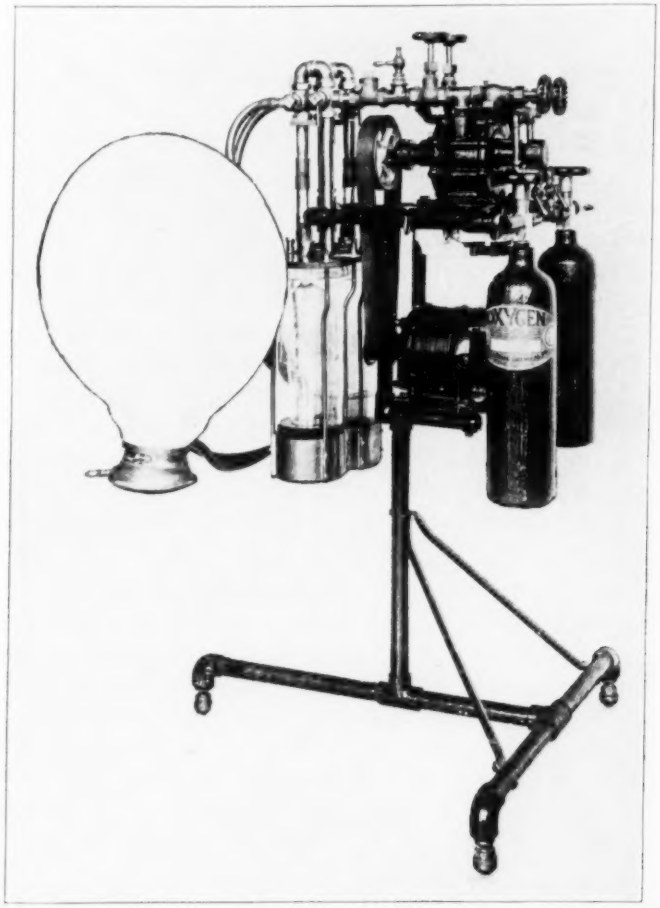


Fig. 1.—Nitrous oxide apparatus with large bag as used for experimental purposes. (For discussion, see text.)

$N_2O$ and only a small amount (experimentally I have estimated that from 1½ to 3 gallons will be necessary for a man weighing 160 pounds) is required to saturate the blood sufficiently to produce anesthesia. When a given amount of $N_2O$ is injected into the closed system and breathing bag, the animal, whose lungs virtually form a part of the closed system, will at once begin to absorb $N_2O$ into its blood from the pulmonary alveolar walls. This absorption goes on until an equilibrium in the quantity of $N_2O$ contained in the animal's blood and tissues on the one side and that contained in the breathing bag and tubes on the other

is established. It is important, however, to remember in this connection that the affinity of the blood and presumably of the central nervous system is greater for $N_2O$ than is the affinity of water for $N_2O$. So far as is known at present $N_2O$ does not form any special chemical combination with the blood or other tissues of the body. It is apparently held in solution in the blood and tissues in the same way as any other indifferent gas dissolves in a liquid, i.e., in direct proportion to the partial pressure exerted by the gas on the liquid. The lipoid content of the blood and central nervous system is generally considered to account for the increased solubility of the gas in these tissues over that in water.

The breathing bag and face-piece shown in Fig. 1 are also modified considerably from that which I first used. The bag shown here I have found well adapted for experimental observations. It is chiefly from this standpoint that I want to discuss the action of nitrous oxide in this article. This bag holds three gallons, but the sides of the bag are flat and when not in use fall together as the air or gas passes out. When in use only about one or two gallons of air or gas need be injected into the bag and this permits the subject to have a full and free opportunity to breathe in any way he pleases. It is desirable that no excess pressure, as from an overfilled bag, be introduced to embarrass the breathing of the subject. The excess amount of work which may thus be easily thrown on the respiratory apparatus in the course of an hour may be astounding, as a brief mathematical calculation will readily show. And the problem is still further complicated both directly and indirectly by the embarrassment to the heart and lung circulation which the amount and peculiar application of this excess work involves. A further feature to be noted in the face-piece is the large opening, about three and one-half inches in diameter, which connects the bag to the air cushion resting on the face. Thus the subject breathes almost directly into the large flexible bag and obstruction of the respiration is reduced to a minimum. While this bag and face-piece serve very well for experimental observations, there are certain objections which may be made to them from a practical standpoint. The first of these is the difficulty of making an air-tight contact between the subject's face and the rubber cushion on the face-piece. The second is the inconvenience of having the large bag near the patient's head. At present, however, I wish to avoid any extensive discussion of the clinical side of this subject.

### THE GASES INVOLVED IN NITROUS OXIDE ANESTHESIA.

The pharmacological relations of at least four gases must always be considered in nitrous oxide anesthesia. These are $N_2O$, oxygen, $CO_2$ and nitrogen. And it may be worth while to remember that a small amount of argon, neon, crypton, xenon, etc., are also present. Ordinarily these gases are supposed to be inactive in the animal organism, but under the peculiar conditions established in the gaseous content of the body under nitrous oxide anesthesia, I am inclined to believe that the presence of these substances, at least at the beginning of the anesthesia, should not be entirely forgotten.

It should be emphasized that the method which I have here used permits investigation of peculiar gaseous relationships which no other device heretofore employed for this purpose could well reveal. For by this method the supply of the four gases ($N_2O$, $CO_2$, N, and O) concerned in the animal's respiration,

may be separately and independently controlled. The $CO_2$, of course, is eliminated by the animal, but it may be allowed to accumulate in the breathing bag for experimental purposes and its relative action in combination with the other gases thus studied. The relative effects of $CO_2$ and O in ordinary forms of breathing and in asphyxia have been thoroughly studied by numerous investigators.[2] When, however, nitrous oxide is introduced, the conditions are very materially changed and only a small amount of work has been done on this phase of the problem.

It was supposed by Sir Humphrey Davy that nitrous oxide was decomposed in the body which thus became flooded with an excess of oxygen which was promptly changed to carbon dioxide. This carbon dioxide then acted as a depressant and caused the anesthesia. It was later shown that nitrous oxide was not thus broken down but was excreted by the lungs in the same form as that in which it had been absorbed. The theory then became prevalent that nitrous oxide acted solely by excluding oxygen from the tissues and that its action was chiefly a matter of asphyxia. That asphyxia may, and in practice certainly does often play a considerable part during the production of the anesthesia, no one at present doubts. But it has been thoroughly established that nitrous oxide possesses distinct specific depressant powers of its own on the central nervous system. In 1897 Kemp[3] published a series of observations on the gaseous content of the blood during nitrous oxide anesthesia. He drew off blood from the femoral artery of dogs anesthetized with various mixtures of $N_2O$ and air and of $N_2O$ and O, and found that complete anesthesia could be produced by the gas when the blood contained quantities of oxygen fully capable of maintaining consciousness and of carrying on the ordinary process of metabolism. When nitrogen was substituted for the $N_2O$, the percentage of oxygen breathed remaining the same, then the anesthesia gradually passed off and the animal regained consciousness. And it has been found by the last Sir Frederic W. Hewitt[4] that a mixture of nitrous oxide 80 per cent and oxygen 20 per cent (the amount present in air) is fully capable of producing anesthesia in suitable subjects. These observations prove beyond doubt that $N_2O$ possesses specific depressant powers on the central nervous system. It has also been shown by Kemp as well as by others that under $N_2O$ anesthesia the $CO_2$ content of the blood is greatly reduced below the normal. But in most cases, however, it has been found that the oxygen content of the blood is reduced in even still greater degree below the normal than is the carbon dioxide. As ordinarily administered $N_2O$ causes the nitrogen (and presumably the argon, etc.) contained normally in solution in the blood and tissues to be rapidly washed out of the system. Kemp's blood analyses for the dog show in several experiments a complete absence of nitrogen from the gases drawn off by the vacuum pump. It is to be noted that in all other forms of anesthesia the nitrogen (about 1.7 vol. per cent) remains dissolved in the blood. *Does the absence of this supposedly inactive gas in any way affect the anesthesia?* In many instances I have observed dogs going under the influence of $N_2O$ in which it appeared to me very probable that the elimination of this nitrogen was essential to the production of successful nitrous oxide anesthesia. It is, unfortunately, extremely difficult to prove this point. For one must, as a general rule, empty out most of the air (nitrogen) from the apparatus (and lungs and tissues

of the animal) in order to fill this space with nitrous oxide so as to be able to obtain a sufficiently high percentage of the gas to produce the anesthesia. This makes difficult the solution of the question as to whether or not the absence of the nitrogen in any way influences the nature of the anesthesia.

In most forms of nitrous oxide apparatus used heretofore breathing had to be carried out under a greater or less degree of pressure. It is interesting to consider what influence, if any, this may have in tending to dam back the $CO_2$ produced in the tissues. While this gas did not apparently accumulate in large quantities in the blood in the analyses made by Kemp, still one is inclined to suspect that the tissues may have been trying to form the ordinary amounts of the gas but were either unable to do so or else they could not pass it over to the blood. And any such accumulation of $CO_2$ in the tissues may very well influence the nature of the anesthesia produced. And similarly any of the immediate precursors of $CO_2$, if allowed to accumulate in the tissues or blood, may affect the character of the anesthesia produced.

### THE SYMPTOMS PRODUCED BY NITROUS OXIDE.

I have studied this topic both from the standpoint of animals and from that of man.

A frog placed in an atmosphere containing a high percentage (90% to 98%) of $N_2O$ becomes well anesthetized in from three to four minutes. When again placed in fresh air the animal fully recovers in about one minute. Profound anesthesia is readily obtained.

The symptoms in dogs vary greatly with the animal and the method of administration. Fig. 2 shows a record of the respiratory movements in a dog just beginning to inhale $N_2O$. The animal was lying quietly on the table and made no resistance in any way. The record was obtained by tying a stethograph around the chest wall and connecting it by rubber tubing to a recording tambour. The first part of the tracing shows the normal respiratory movements when the animal was breathing a sufficient percentage of oxygen and the $CO_2$ was not allowed to accumulate to excess. At the point indicated $N_2O$ was run into the breathing bag and shortly thereafter the depth of the respiration began to increase. This is mainly due to the action of $N_2O$. It is the typical effect of this gas on the respiration. There is one other point to be considered in the experiment, however, and that is the fact that in this case when the $N_2O$ was run into the bag then the oxygen which the bag contained was considerably diluted. This would also cause the animal to breathe more deeply. But independently of this dilution of the oxygen, the first effects of nitrous oxide in sufficient concentration appear to be to stimulate the respiratory center. After a time, as the animal passes more fully under the influence of the gas, the depth of the respiration decreases while the rate varies somewhat, but on the whole is accelerated beyond the normal. This does not seem to be due to any accumulation of $CO_2$ in the breathing bag or apparatus, for it is very easy to wash out the $CO_2$ as fast as it is formed. The animal usually does better, however, if a certain amount of rebreathing and $CO_2$ accumulation is permitted.

These same phenomena occur in the human subject. It is very interesting to experience the beginning action of the gas. If one fills the bag partly full of

oxygen and breathes this for a while (washing out the $CO_2$), he may at first note a very slight sense of fullness in the head and possibly there may be a feeble flushing of the skin especially of the face and neck. Whether this is due to a slight $CO_2$ accumulation in the lungs (dead space, etc.), caused by the small amount of obstruction to the normal respiration, or is due entirely to excitement or the mere feeling that one "expects something" I have not been able to determine. It is of but little consequence, however, and soon passes off as one adjusts himself to breathing into and out of the bag. Slight odors, as from a new rubber bag, or of oil from the pump, etc., sometimes cause one to be a little apprehensive. And the mere act of fixing the attention on the respiration is sufficient to cause certain minor variations in most subjects. When the $N_2O$ is turned on, however, there is an immediate feeling of ease in breathing. The sensation can best be compared to the effect of oiling a new machine. One is somewhat surprised how readily he can breathe deeply and fully and without special exertion. This sensation does not occur if one instead of running nitrous oxide into the bag, should fill it to a corresponding degree with oxygen. I have been inclined to believe, therefore, that it is due to a direct stimulation of the respiratory center by the $N_2O$. I have considered the question of whether or not the processes of diffusion of the gases in the lungs, or the rate or ease of absorption or excretion of the oxygen or $CO_2$ through the alveolar epithelium might be influenced in any way by the presence of $N_2O$ rather than of nitrogen. I have not been able to reach any conclusion on these matters. It seems probable that certain obscure changes are produced in the metabolism of the tissues on account of the markedly subnormal $CO_2$ and oxygen content of the blood, as shown by Kemp. It would be interesting to know whether or not these low percentages of $CO_2$ and oxygen persist in the blood in those cases in which anesthesia is produced by approximately 80 per cent $N_2O$ and 20 per cent oxygen as in Hewitt's experiments. I have occasionally believed that in rare instances in dogs which were fully anesthetized I could raise the percentage of oxygen in the bag to perhaps 30 per cent without allowing the animal to revive. In this case, of course, while I might markedly increase the percentage of oxygen in the bag, I did not correspondingly lessen the amount of $N_2O$ in the animal and in the apparatus. In this feature there is a great difference between the apparatus which I have here used and most other forms of nitrous oxide machines, for in these if the amount of oxygen administered is increased, this generally means a corresponding diminution of the amount of $N_2O$ given with correspondingly increased chances for variations in the character of the anesthesia.

I have noted only occasionally, as have a number of my students, that just as one begins to breathe a fairly concentrated mixture of $N_2O$, there may be detected a faint metallic sweetish taste on the tip of the tongue. The sensation reminds one of the taste of saccharine. In my own case this taste has never lasted for more than a second or two, but one student was able to detect it over a prolonged period. It is probably due to $N_2O$ carried in the blood from the lungs to the taste organs.

It will be noted from Fig. 2 that the animal did not struggle as the gas was administered. In some cases I have seen dogs go quietly to sleep and apparently never be conscious at all that they were being anesthetized. In a gen-

tle animal which is especially susceptible to the gas, this may frequently occur. It is by no means the rule, however, and there is often struggling especially if the animal was excited before the anesthesia was started. It is well known that certain human subjects are especially resistant to the gas and I have frequently found this to be true for dogs. In some cases I have been entirely unable to obtain any true anesthesia at all. In these cases cardiac slowing and other complications nearly always come on as one attempts to crowd the gas. This appears to be partly due to stimulation of the vagus center in the medulla for section of the vagi usually accelerates the heart and this is generally even more marked after atropine. I have seen three or four especially striking cases of

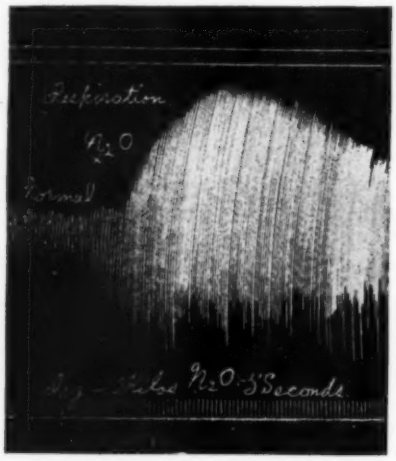


Fig. 2.—This tracing shows the respiratory movements of a dog to which (at X) nitrous oxide (plus oxygen) was administered. There is a slight exaggeration of the respiration because the animal had been breathing mainly with its diaphragm before the gas was given, but after this the chest movements became much increased. The stethograph recorded more of the thoracic movements than it did of the abdominal.

this kind. An animal which is excited or struggles is much more liable to manifest these cardiac symptoms. After atropine the animal usually takes the gas considerably better, indicating better aeration of the blood in the lungs from the improved circulation. The slowing of the heart may be very marked and apparently may be the cause of death in some cases. I have not taken string galvanometer tracings of the hearts of these animals, but this would be instructive. It has seemed to me in one or two instances that peculiar arrhythmical contractions were set up in the heart and that this finally ended suddenly either with complete stoppage of the heart, or with the establishment of a condition resembling heart block. The cause of these reactions is not at all clear. One would suspect a lack of oxygen, or $CO_2$ poisoning, but when the $CO_2$ is well washed out of the gases breathed and the conditions are the same as those under which other animals have been well anesthetized, then one is inclined to look for a difference in the animals. I suspect that very nearly, if not quite this same thing, may have occurred in a few instances in man.[5] For that reason I wish to refer briefly to Fig. 3 which is a tracing of the apex beat of a dog which was given nitrous oxide. The animal was not a good subject, but was finally apparently well anesthetized. After the anesthesia had continued for perhaps half an hour,

the pulse in the femoral artery became irregular and finally stopped rather suddenly. With considerable difficulty the animal was revived by means of intermittent compression of the chest. But when the respiration was restored, the animal did not promptly regain consciousness and remained in a semicomatose or somnolent condition for two or three hours. It was noticed about five hours after the animal revived that the heart was irregular and the tracing here shown was made. The animal improved and in about a week the heart had apparently returned to normal. The animal was kept for forty days thereafter, but no further cardiac disturbance was observed.

It seems evident to me that the human subject must be very much more susceptible to nitrous oxide than is the average dog. The anesthesia in all cases is of a much lighter form than that produced by ether. In dogs it is as a rule impossible to destroy the corneal reflex, for in the deepest anesthesia in these animals the slightest touch of the cornea or eye lid or even eye lashes causes immediate winking. The eyes remain open and keep up peculiar rolling or staring movements so that one often wonders whether or not the animal is fully anesthetized. If the gas be removed suddenly, however, the animal wakes up and

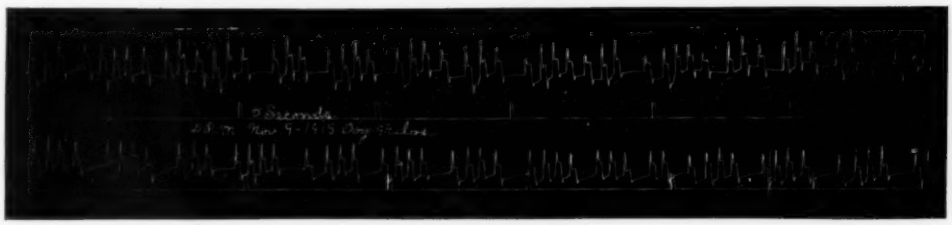

Fig. 3.—Tracings of the apex of the heart in a dog in which under nitrous oxide anesthesia cardiac irregularities developed. For a full description see text. This tracing was taken about five hours after the animal had been anesthetized. At this time it was observed by Mr. John A. Higgins that the animal was in a dazed or semicomatose condition, and that the heart beat was very abnormal. The record here shown was then made.

stares about in a way which shows that it had been completely unconscious. During the anesthesia the pupils are dilated but the light reflex is preserved.

### THE ACTION OF NITROUS OXIDE UNDER VARYING CONDITIONS.

If pure $N_2O$ be inhaled, unconsciousness results in a period of from thirty to sixty seconds. But if oxygen be added to the inhaled gas, the time required to produce unconsciousness rapidly increases as the oxygen rises from zero up to five, ten or more per cent. With more than ten or twelve per cent oxygen content mixtures of nitrous oxide and oxygen usually produce unconsciousness only after considerable periods or not at all, depending on the patient. There seem to be great variations in this respect, however, in the human subject, and I have noted a similar reaction in animals. I should like to emphasize this point in particular since it has a direct bearing on the administration of nitrous oxide by the method which I have here used.

It will be noted by reference to Fig. 1 that there is a considerable "dead space" in the apparatus. The wash jars, tubes, pump, etc., and the breathing bag all represent space which in the beginning contains air. When the animal is connected to the apparatus then its lungs also add "dead space" to the system.

This "dead space" contains oxygen and nitrogen. The oxygen can be readily used up by the animal, but the nitrogen must be gotten rid of. The amount of this nitrogen depends on the construction, size, etc., of the apparatus and on the size of the animal. The blood (and tissues) of the animal also contain about 1.7 per cent of nitrogen which presumably diffuses out into the lungs and is then breathed out when nitrous oxide and oxygen are administered. It is necessary to remove a large part of this nitrogen from the apparatus to secure the best success. This is done by filling the bag partly full of nitrous oxide and running the pump for a while. The animal breathes the mixture of air and $N_2O$ and absorbs a portion of the gas, while at the same time some of the nitrogen in its blood is breathed out. The sodium solution and the sulphuric acid in the wash jars also absorb some nitrous oxide. In a little while the bag is emptied out into the air. This is accomplished by opening a valve on the right (positive) side of the machine while the pump is running. In one or two seconds the bag can be emptied as much as desired and then more $N_2O$ is run in until the bag is about one-haif or two-thirds full. This is repeated about three or four times as a rule with dogs. One should not hurry this process. It usually takes at least five minutes to anesthetize a dog deeply and any attempt to crowd the gas faster generally excites the animal and does not improve the anesthesia. It is better to proceed slowly and allow the animal's blood to become as nearly saturated as possible for each concentration of the $N_2O$. In this manner the action of the drug is brought on slowly and in a perfectly successful experiment the animal may be fully anesthetized apparently without being conscious that anything unusual is occurring. Often it is not necessary to give any oxygen until the animal is anesthetized, for the oxygen in the apparatus and in the lungs, etc. ("dead spaces") serves to keep the animal in good condition for some time. When needed, however, more oxygen should be injected.

It may seem that five minutes is an unreasonably long time to require for the production of nitrous oxide anesthesia. We should remember, however, that much more time than this may be required with ether, etc., and when we think of anesthetizing an animal in thirty to fifty seconds with nitrous oxide it is interesting to consider the possibility of doing this same thing with chloroform or ether vapor which are exceedingly well absorbed by the blood. And it is probable that in any rapidly produced nitrous oxide anesthesia there may be a considerable element of asphyxia which is undesirable.

In this connection I should like to refer to some physiological experiments[6] on respiration which involve certain features usually concerned in nitrous oxide anesthesia.

"1. *The Immediate Effects of Total Rebreathing (Due Chiefly to Excess Carbon Dioxide).*—The nostrils are compressed with a nose-clip and the subject breathes from and into a rubber bag containing 20 to 40 liters of air. The amplitude of respiration is soon augmented, and in the course of a few minutes the subject is panting heavily forty times a minute. He usually develops a typical carbon dioxide headache, but this wears off in fifteen or twenty minutes after the experiment is ended." These results are produced by breathing for a *"few minutes"* into a closed bag. If in addition to these effects, which are due chiefly to carbon dioxide accumulation, there be added the further effects of oxygen

want which are usually present from the very beginning in the administration of nitrous oxide, what will be the results of these purely physiological phenomena when complicated by the addition of nitrous oxide in those forms of apparatus in which rebreathing into and out of a closed bag is carried on for considerable periods of time?

"2. *The Effects of Insufficient Oxygen Without Excess of Carbon Dioxide.* —The above mentioned bag is refilled with 20 to 40 liters of fresh air and the experiment performed again, but with this difference, that a vessel of 1 or 2 liters capacity filled with soda-lime or broken sticks of sodium hydrate is placed between the bag and the subject's mouth so that he breathes through it into and from the bag. The carbon dioxide exhaled by the subject is thus absorbed, and he gradually consumes the oxygen in the bag. As a rule there is *no noticeable deepening or quickening of the breathing,* and the subject will first become cyanosed and then unconscious without appreciable augmentation of breathing. This experiment should *always be carefully supervised,* as it is not free from danger. If continued for more than ten minutes, it is usually followed by a severe frontal headache, developing slowly for several hours thereafter, together with other ill effects and lasting from twenty-four to forty-eight hours." It is particularly interesting to consider this experiment in connection with those forms of nitrous oxide apparatus in which the patient inhales the gas (plus a varying but usually small amount of oxygen) from a tank or reservoir and then exhales out into the open air. In these machines the carbon dioxide is probably fairly completely removed as fast as it is exhaled from the lungs. The small percentage of oxygen usually given (e.g., from two to ten or twelve per cent) with the nitrous oxide may cause a rather close simulation of the conditions established in the above experiment in which *cyanosis* and *unconsciousness* may be produced *without any anesthetic.* I should like to give one further quotation bearing on this point from Haldane and Poulton.[7] * * * "Still more sudden exposures to anoxemia occur when air containing little or no oxygen is breathed; for in this case the oxygen previously present in the alveolar air, and even in the venous blood, is rapidly washed out; the result is that consciousness is suddenly lost *without evident preceding hyperpnœa,* although abundance of $CO_2$ is present in the arterial blood. Haldane and Lorrain Smith observed sudden loss of consciousness after 50 seconds on breathing air which was afterwards found to contain 1.8 per cent of oxygen. During any exertion the loss of consciousness is still more sudden. Thus it is a common experience with miners going into an atmosphere of nearly pure fire damp ($CH_4$), or climbing up so that their heads are in the gas, that they drop suddenly as if they were shot."

I do not care to discuss this point further, but may state briefly that my own experiments, together with the results obtained by others, have led me to conclude that it is impossible to obtain a rapid (1 minute) production of anesthesia and unconsciousness in dogs with nitrous oxide and oxygen at atmospheric pressure unless the oxygen content of the mixture is so low that the loss of consciousness is due *almost entirely* to the lack of oxygen. Presumably, with certain modifications, this is true in the human subject also. On the other hand it seems probable that in all dogs which do not possess a special idiosyncrasy against the gas, mixtures of nitrous oxide and oxygen containing sufficient amounts

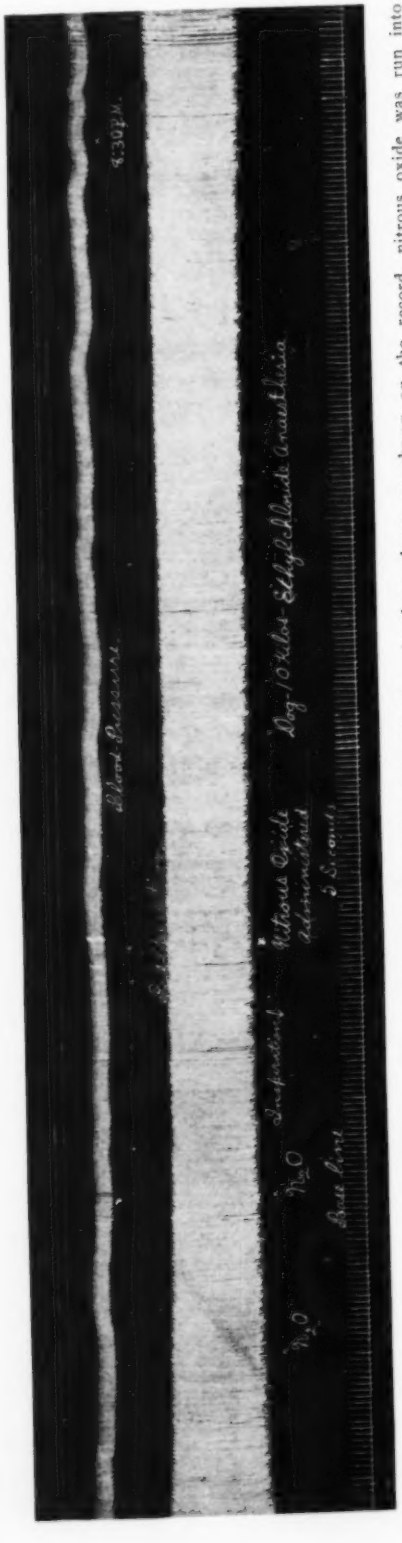


Fig. 4.—Blood pressure and respiration in a dog anesthetized with ethyl chloride. At three places, as shown on the record, nitrous oxide was run into the breathing bag. This was done in order to observe the effects of the gas on the circulation and respiration. The results were practically nil so far as can be observed from the record.

of the latter to avoid most if not all asphyxial effects, may be used to produce anesthesia *provided sufficient time be allowed for the gas to act and the $CO_2$ be completely removed as fast as it is excreted by the lungs.* If a high per cent of oxygen is used, anesthesia cannot be quickly produced but asphyxia may be avoided. The time required may be considerable, perhaps from five to fifteen minutes or longer. But as the tissues gradually become more and more saturated with the gas, there will be a gradual depression of the central nervous system which will finally result in unconsciousness.

It was long ago observed by Goldstein[8] that anesthesia appears more quickly and with a proportionately less degree of asphyxia, the higher the organization of the brain—namely, earlier in man than in laboratory animals. I have been able to confirm this observation many times. And in addition the anesthesia appears as a general rule to be deeper in man than in dogs, although in some ani-

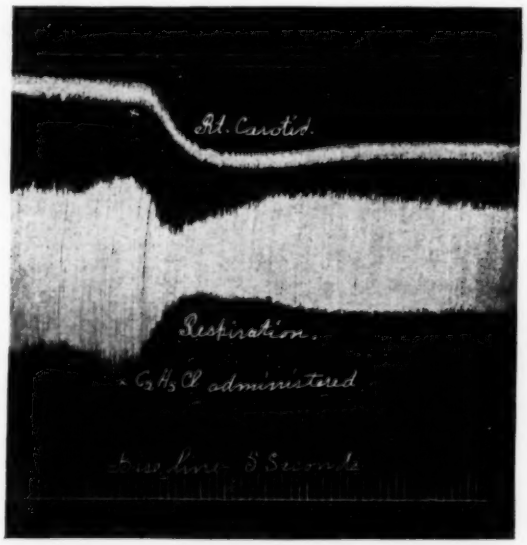


Fig. 5.—This animal was anesthetized with nitrous oxide. At the point indicated ethyl chloride was injected into the bag. There is an immediate fall in pressure and the respiration is much diminished.

mals a profound anesthesia may be readily obtained if all carbon dioxide effects be carefully avoided.

It seems probable that in average cases the heart and circulation are not much affected by the gas. Fig. 4 shows the result produced by injecting nitrous oxide into the breathing bag when the animal was already anesthetized by ethyl chloride. Three injections were made but the effects on both blood-pressure and respiration were practically nil. This corresponds very well to the injection of an ordinary drug solution into the femoral vein when an animal is anesthetized with ether. (Fig. 5 shows the reverse of this experiment and illustrates the action of ethyl chloride on an animal already anesthetized by nitrous oxide.) As a kind of check on these experiments another tracing (Fig. 6) is shown in which at two places a small amount of carbon dioxide was injected from a tank into the breathing bag. There is an immediate stimulation of the respiration and the blood pressure falls, probably from a direct action on the heart. The gas was

quickly emptied out and the bag was again refilled with nitrous oxide plus a suitable amount of oxygen. This shows quite well the action of even small amounts of carbon dioxide. I strongly suspect that some such action as this, either by excess of carbon dioxide, or from lack of oxygen, or both, constitutes the real cause of the undesirable after effects which are liable to follow from prolonged nitrous oxide anesthesia. And I am inclined to believe that these after effects may be very generally avoided by a correct and scientific administration of the nitrous oxide.

I have repeatedly observed, as have others, independently in my laboratory, that if one breathes a mixture of nitrous oxide and oxygen for a certain time, for example five minutes, and then passes under the influence of the gas to a given degree, he can then considerably increase the quantity of oxygen in the bag without lessening the influence of the nitrous oxide so far as the subject of

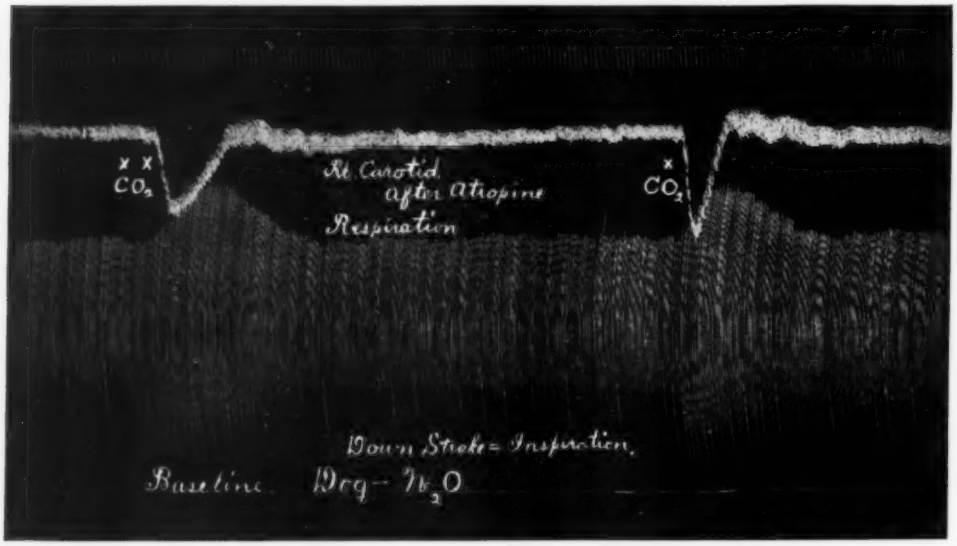


Fig. 6.—This tracing was made in a class experiment by Messrs. Mitchell, Day, Lueking and McKee. It shows the results produced on the respiration and blood pressure by injecting (twice) a small amount of carbon dioxide into the breathing bag while the dog was anesthetized by nitrous oxide. In each case the $CO_2$ was quickly emptied out of the bag after the animal began to show marked symptoms.

the experiment himself can determine. The reason for this appears to be as follows: On breathing the $N_2O$ at first the whole body of the subject after a time becomes saturated with the gas at the given partial pressure. (As the first portion of gas is absorbed, one can see the bag shrink fairly rapidly with animals.) More $N_2O$ must be run into the bag to replace that absorbed. But after the anesthesia or analgesia has reached a given degree, then if no more gas, but only oxygen, is given, the effects of the $N_2O$ on the subject should remain fairly constant. It will be noted that the gas is excreted only into the bag from which in a given time approximately the same quantity of $N_2O$ will pass back again into the blood. Supposing the bag was filled to the amount of two gallons* with 90 per cent $N_2O$ and 10 per cent oxygen. If then one adds a quart of oxygen to the bag the per cent of oxygen the patient would breathe should be increased by

one-ninth of the total amount of mixed gases in the bag after the quart of oxygen is added. It would appear that this oxygen should be readily absorbed by the lungs in approximately the same proportion and quantity as oxygen is absorbed by the blood from the air (which contains oxygen in about the same proportion as the bag would now contain it, i.e., about 20 per cent). This would probably not be quite correct, for nitrous oxide has some power to displace oxygen from its solution in water (Sir Humphrey Davy[9]), and this probably holds good for the blood in the pulmonary capillaries also. On the other hand, when the quart of oxygen is run into the bag, the latter will be expanded by a volume equal to one quart and into this space the nitrous oxide already in the bag and also that

Fig. 7.—The animal was anesthetized with nitrous oxide. At the point indicated adrenaline (½ c.c., 1-10,000) was injected intravenously. The vagi were intact. (For discussion, see text.)

dissolved in the blood and tissues of the subject, may diffuse. But if, for example, the blood and tissues of the subject had absorbed two gallons of $N_2O$ and the bag contained two and one-fourth gallons of (mixed) gases after the quart of oxygen was added, then there would be a chance for the $N_2O$ to be diluted by approximately one-seventeeth of the total volume of gases or 5.8 per cent. At that time the subject might be breathing almost 20 per cent of oxygen and this is readily absorbed by the hemoglobin of the blood. In other words, the relative increase in percentage of oxygen breathed when a given amount of oxygen is added to the bag, is greater than is the relative amount of dilution of the nitrous oxide with which the subject is saturated after the oxygen is added to the bag.

It was shown by Van Arsdale[10] in 1891 that the breathing of nitrous oxide to and fro from a bag in which the gas (plus the desired amount of oxygen) was contained at an increased pressure above that of the atmosphere caused an increase in the depth of the narcosis produced. (This was an entirely different principle from that which Paul Bert[11] and later Claude Martin[12] used in which the patient or animal was placed in an air-tight room, the air pressure in which was raised one-fourth above that of the atmosphere after which 80% $N_2O$ plus 20% oxygen was administered to the patient or animal.) I have tried to verify Van Arsdale's results many times. In some cases (with dogs) increasing the pressure of the gas in the bag does deepen the anesthesia, but in many other cases I have not been able to demonstrate any advantage from this increased pressure.

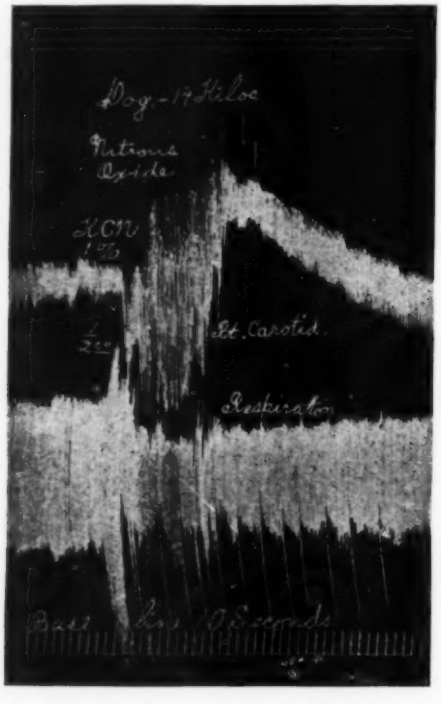


Fig. 8.—Dog anesthetized with nitrous oxide. At the point indicated ½ c.c. of 1% KCN was injected intravenously. The vagi were intact. (For discussion, see text.)

Perhaps the increased respiratory exertion, the marked hindrance to the pulmonary circulation and the attendant obstruction of gaseous exchange in the lungs were sufficient in many cases to overcome the advantages of the increase in absorption of the $N_2O$ which the raised pressure might bring about.

The intravenous injection of adrenaline in an animal under nitrous oxide anesthesia gives a practically normal reaction, i.e., the record is almost exactly like that produced by adrenaline in an animal under ether. The rise in pressure here probably supplies more oxygen to the brain and whatever asphyxia may have been present from the administration of the nitrous oxide is thereby reduced. (See Fig. 7.) On the other hand, the injection of cyanides (which are supposed to cause an internal asphyxia by lessening the tissue oxidations through

inhibiting ferment action) causes a markedly increased reaction both as regards the respiration and the circulation. The animal also shows a more marked convulsive reaction than it does under ether. (See Fig. 8.) I have controlled this by anesthetizing the animal first with $N_2O$ and obtaining records of the blood pressure and respiration from the cyanides and then giving the animal ether, after which more records were obtained.

The motor areas are much more sensitive under nitrous oxide than under ether. One can easily secure very extensive movements of the muscles of the opposite side and can readily pick out the areas for individual groups of muscles. I have observed that dogs under nitrous oxide anesthesia may not well withstand extensive operations, particularly if the abdomen is opened and the viscera manipulated in any way.

In several respects there is a striking similarity between the effects of nitrous

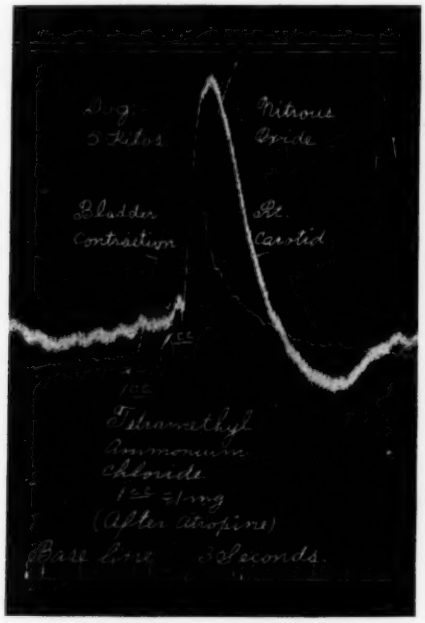


Fig. 9.—Dog anesthetized with nitrous oxide. The tracing shows the blood pressure (Rt. carotid) and the bladder contractions (up-stroke). At the point indicated 1 c.c. of tetramethylammoniumchloride was injected intravenously. The animal had previously received 1½ mgs. of atropine.

oxide and those of morphine in dogs. Among these may be mentioned the production of Cheyne-Stokes respiration. This is generally present in prolonged anesthesia in dogs. The irritability of the cord is also much less depressed than is the case with the methane series of anesthetics and this action also closely resembles that of morphine. As under morphine defecation also sometimes occurs, but I have generally been inclined to attribute this to asphyxia, although other factors may be involved. A peculiar feature is often noticed in the fact that the dogs, while lying quietly and apparently fairly well anesthetized, may be aroused and waked up by stimulation or shaking in a manner very similar to

*For clearness of description I have assumed that the *volume* of one gallon of the gas may be considered equal to the volume of four quarts. We need not consider variations of temperature, pressure, etc.

that possible under a moderate dose of morphine. When thus aroused there is also often observed a marked acceleration and increase in strength of the heart beat. If the animal be again left alone it will soon return into the somnolent, or perhaps analgesic state, very much as occurs after morphine. It is difficult to study the analgesic effect of nitrous oxide separately and apart from the production of total unconsciousness in dogs, for these animals, so long as they are conscious, are very likely to struggle and try to escape even though they feel no pain whatsoever.

The thought has occurred to me many times that nitrous oxide might be used as a hypnotic. By ordinary methods of administration this is obviously impractical. But by a slight modification of the apparatus which I have used I am inclined to believe this idea might be very well put into practice. I have tried

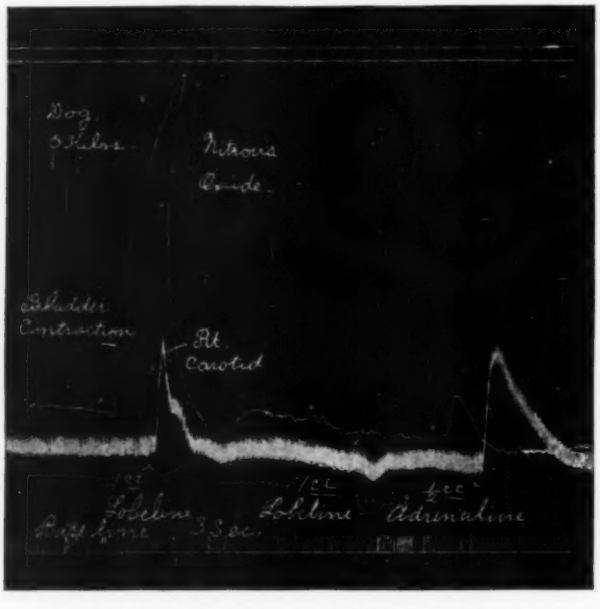


Fig. 10.—Dog under nitrous oxide anesthesia. Bladder contractions (up-stroke) and blood pressure. At the point indicated (on the left) 1 c.c. of lobeline was given intravenously. A marked contraction of the bladder and a small rise in blood pressure were produced. A little later a second dose of 1 c.c. of lobeline was given. Almost no results were produced by this, showing that the first dose of lobeline had produced ganglionic paralysis. Later a small dose (½ c.c.) of adrenaline was injected. This gave a slight bladder contraction and a small rise in blood pressure.

repeatedly to compare the mild on-coming effects of the gas as breathed with a considerable proportion of oxygen with the physical and mental sensations present as one begins to fall asleep. There is a very striking similarity, a marked feeling of tiredness and exhaustion, the limbs feel heavy and the eyelids tend to close. One's mentality gradually sinks and there is difficulty in maintaining connected thought. The natural inclination of the subject of the experiment is to lie down quietly and fall asleep. The sensations remind one of the feelings of a child worn out by a long day's play when it lies down at night to sleep. Sometimes I have noted slight muscular twitchings or feeble jumping or convulsive moments. These would probably not occur if the gas were administered very slowly with plenty of oxygen and a sufficiently long period of time were used

to bring on the action of the drug. Suggestion appears to play a noticeable part in this action, for if one keeps perfectly quiet and at rest and tries to go to sleep, then the somnolent action of the gas is especially liable to be well marked. It would appear that this matter of suggestion extends even to dogs. For an animal which is petted and induced to lie down quietly and at complete rest may very often take the gas readily and peacefully fall asleep.

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# OBSERVATIONS ON CHOLESTERIN RETENTION AS A FACTOR IN CELL-PROLIFERATION*

By Georgine Luden, M.D., Rochester, Minn.

THE influence of heredity, the cancer age, the relatively large percentage of recurrence in malignant conditions, and lesions produced by trauma are recognized by the medical profession throughout the world as important contributory factors in malignant cell-proliferation. It is well known, for example, that in certain persons a slight injury will result in the formation of a rapidly growing tumor—a kick during a football game may be followed by sarcoma,[1] the irritation from the ragged edge of a tooth, by inoperable carcinoma. In these cases, however, a mere spark, as it were, causes a conflagration. The injury itself may be considered only a provocative factor, not the real cause, in the formation of the malignant growth; sparks do not create a conflagration unless they fall where inflammable material has accumulated.

That the fundamental factor in malignant diseases, whatever it may be, is widely distributed throughout the organism seems evident from the literature reporting conditions in which more than one tissue has become involved in the process of degeneration. Such cases, although not frequent, are by no means exceptional. Bartlett[2] concludes that "multiple primary malignant tumors occur in approximately 0.2 per cent of all cases of malignant tumors."

In this connection attention should be drawn to the fact that the term "multiple primary tumors" has been used in the literature repeatedly without distinction for the simultaneous occurrence of (a) different types of carcinoma, (b) carcinoma and sarcoma, and (c) tumors of totally diverse histologic character. The relative frequency of these three varieties of multiple types of malignant growths may be indicated as follows:

1. *Carcinoma Duplex.* (A term originally used by de Vries[3]): conditions in which two types of carcinoma, or carcinoma and epithelioma occur simultaneously;

2. *Carcinoma and Sarcoma:* conditions in which malignant degeneration is observed simultaneously in both epithelial and connective tissue;

3. *Multiple Type of Primary Malignant Tumors:* conditions in which tumors of a totally diverse histologic character are found simultaneously. These are comparable to those reported by Walter[4] and Hansemann,[5] and are not to be confounded with the class of malignant neoplasms known as teratomas.

As far as can be ascertained, 119 cases of carcinoma duplex, 41 cases of carcinoma and sarcoma, and 15 cases of the multiple type of primary tumors have been observed in man and reported in literature, including the cases reported by Harbitz.[6] In addition, I have studied 2 cases of carcinoma duplex, 3 cases of carcinoma and sarcoma and 2 cases of the true multiple type of neoplasms. One of the latter has been reported previously.

Seven cases of carcinoma and sarcoma in rodents have been reported by

*From the Mayo Clinic, Rochester, Minn.

Woglom,[8] Haaland,[9] Russell,[10] Loeb[11] and Nicholson,[12] and 2 cases of the spontaneous multiple type of primary tumors in dogs have been described by Bartlett. It must be especially emphasized that although Bartlett suggests the possibility that the etiology of the types of tumors found in each of his two dogs was different, "one being indirectly due to a metabolic disturbance, the other, the mixed tumor, being in some way connected with an embryonal anlage," none of the primary multiple type of tumors in man herein reviewed belong to the class known as teratomas.

Two other facts demonstrating the ubiquity within the organism of the fundamental factor in malignant cell-proliferation are the occurrence of malignant degeneration in originally benign tumors, commented on by many authorities, and the "precancerous stage" described by Wilson,[13] MacCarty[14] and others.

As regards the identity of the causative factor, the branches of science that seem most particularly well fitted for the investigation are bacteriology, parasitology, and chemistry. Bacteriology thus far has been unable to furnish any material evidence of bacterial origin of malignancy. Parasitology has yielded many brilliant "finds," none of which has stood a five-year test. Physiologic chemistry, on the other hand, while it has offered no complete explanation of cancer, has already supplied a number of data indicating that marked metabolic changes occur in and go hand in hand with the progress of malignant disease. Lewis and Benedict[15] have called attention to the increase of the sugar content of the blood in carcinoma. Davis[16] describes a hema-uro-chrom test giving valuable information in cases of malignant disease, which he does not claim to be specific for cancer, but considers indicative rather of an increased hemolysis, cytolysis and proteolysis. The nitrogen content has been studied by many observers and found increased both in the urine output and in the tumor-tissue (Robin,[17] Mueller, Gaertig, Klemperer, Embden, Knoop, Langenstein[18]). Capella[19] has noted a sulphur reaction positive in the urine of patients with cancer. Pentagna[20] has called attention to the presence and importance of glycogen in malignant tumors "which increases in proportion to the degree of malignancy exhibited by the neoplasm."

It has been universally conceded that normal cell-proliferation or growth, is a proliferation of cells *within* normal bounds, and that a neoplasm represents a form of cell-proliferation *exceeding* normal bounds. The question then naturally arises: Is there any chemical substance which appears to be essential for normal proliferation, or growth, the activity of which may be traced in abnormal proliferation, or malignancy?

The following facts, I believe, seem to indicate that such a substance is cholesterin.

Dorée, Ellis and Gardner[21] have shown by chemical analysis that the cholesterin content of the egg-yolk diminishes proportionally to the growth of the chick embryo, cholesterin being used in the process of cell-proliferation and assimilated in such a way that it cannot be recovered in the same quantity from the mass of cells representing the embryo. Aschoff[22] and Autenrieth and Funk[23] have called attention to the increase of cholesterin in the blood during pregnancy. Bacmeister and Havers[24] demonstrated that, whereas the cholesterin content in the blood of pregnant bitches increases until the pups are littered, it gradually

returns to normal shortly afterward. Burnett and Robertson[25] in experimenting with tumor-grafted rats, showed that the intravenous injection of cholesterin-sodium-oleate emulsion causes the tumors in the injected animals to double in size in a given time those in the tumor-bearing controls, all the animals having been grafted at the same time and with the same tumor. Browder[26] has proved that the addition of 0.01 gm. of cholesterin to the culture medium increases the rate of division in paramecia from 1.33 to 5 times, as compared with controls grown in the same culture medium without added cholesterin.

The above observations strongly suggest the existence of some kind of correlation between cholesterin-increase and cell-proliferation, both under normal and abnormal conditions, and appear to indicate not only that cholesterin is associated with active cell-proliferation, but also that it acts as a stimulant to cell division.

Before enumerating other facts based on experimental and clinical data which seem to support the above deduction concerning the physiologic activity

Fig. 1.—(85046.) Malignant growth involving upper third of ulna and radius—osteochondroma sarcoma. Tumor developed within three months at site of injury sustained two years previous. Showing proliferation of the bone. (H.E. × 120 diam.)

of cholesterin as related to cell-proliferation, a few words may be said in regard to the organs that regulate cholesterin metabolism and its elimination. Named in the order of their apparent relative importance they are as follows:

1. Adrenal (Rothschild,[27] Sternberg,[28] Landau,[29] Gardner and Lander,[30] Stewart,[31] Weltmann,[32] Hueck,[33] McMeans[34]).

2. Liver (Rothschild, Weltmann, Anitschkow and Chalatow,[35] McMeans).

3. Spleen and the "endothelial apparatus" (Aschoff and Landau,[36] Soper,[37] Rothschild, McMeans).

4. Ovary and corpus luteum (McMeans).

Apart from these organs of regulation, the body has at its command a number of other means by which, under normal conditions, it can maintain its cholesterin balance. Surplus cholesterin is eliminated, for example, in the feces (McNee[38]) and the bile (Rothschild), and is stored in both the endothelial lining of the blood vessels, as in arteriosclerosis, (Klotz,[39] Saltykow,[40] Anitschkow,

Krylow,[41] McMeans), and in the body-fat (Rothschild, Hueck, Wacker[42]). In addition, there are what might be called "emergency safety valves," such as cholesterin cysts, boils, etc. An instance of the latter occurred in one of our cholesterin-fed goats which developed a boil, the contents of which had a cholesterin value of 0.70 per cent, about four times that of normal goats' blood.

Notwithstanding these natural means of defense against an accumulation

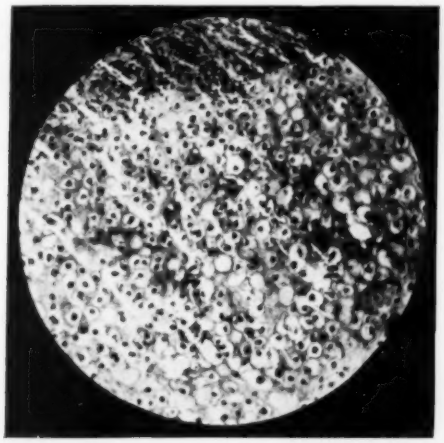

Fig. 2.—(Same as Fig. 1.) Proliferation of cartilage. (H.E. × 250 diam.)

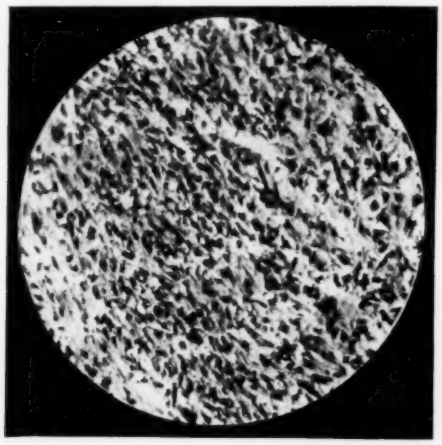

Fig. 3.—(Same as Fig. 1.) Proliferation of connective tissue. (H.E. × 250 diam.)

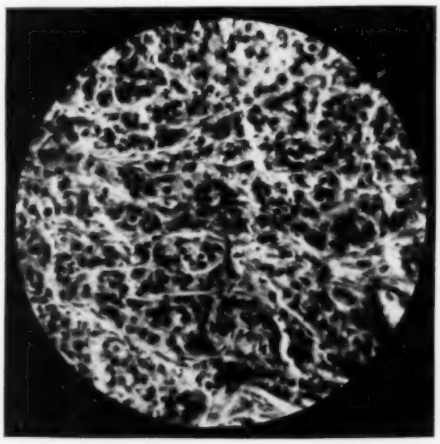

Fig. 4.—(Same as Fig. 1.) Proliferation of epithelium. (H.E. × 250 diam.)

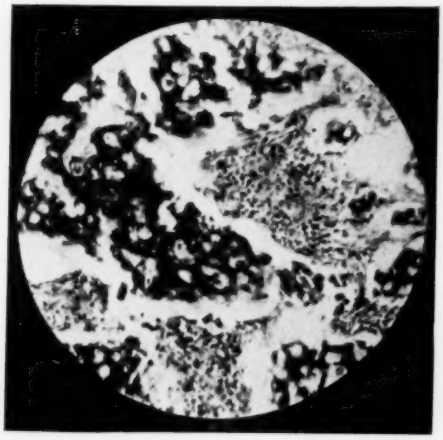

Fig. 5.—(Same as Fig. 1.) Proliferation of the epithelium showing a different type of carcinoma (myxomatous degeneration). (H.E. × 250 diam.)

of cholesterin, there occur at times symptoms which it seems possible to explain only as being due to cholesterin retention and these symptoms go hand in hand with cell-proliferation (Figs. 6 and 7).

A patient (No. 63175), male, aged 57, came to the Mayo Clinic in 1912 on account of "bladder trouble." An operation (prostatectomy) adeno-fibromatous hypertrophy was discovered, but no trace of a malignancy was noted

microscopically. Four years later the man returned in a critical condition and died within three weeks. At autopsy extensive atheromatosis with ulcerating plaques was found in the aorta, together with carcinomatous nodules in the prostatic bed, malignant proliferation of the epithelium and the sebaceous glands in the suprapubic scar. The skin was not broken. It will be remembered that artificial atheromatosis has been produced in animals by prolonged cholesterin feeding (Anitschkow, Saltykow, Stuckey, McMeans) and that the atheromatous plaques were microscopically identical with those found in man.

Proceeding on the working hypothesis that essential factors for the effectual progress of malignant proliferation are,[43] (1) the creation of a disturbance of metabolism which would lead to an accumulation of cholesterin, (2) an extraneous irritant, and (3) the breaking down of the "lymphoid-defence," the importance of which has been established by the work of Murphy and Morton,[44] I began in March, 1915, to give light treatment by rœntgen rays to a double-adrenal-

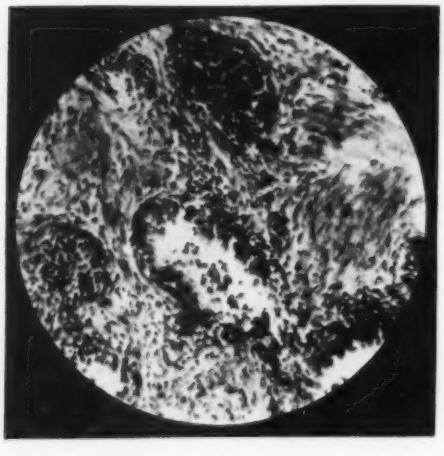

Fig. 6.—(63175.) Recurrent carcinoma of the prostate. (H.E. × 350.)

Fig. 7.—(Same as Fig. 6.) Malignant proliferation of the epithelium and sebaceous glands found at autopsy in operative sinus, four years after operation. (H.E. × 350.)

ectomized spermophile (citellus tridecemlineatus). Experiments conducted by Mann* had already shown that these spermophiles survived double adrenalectomy better than most animals. The results obtained were a marked change in the blood picture, there being an increase of normoblasts from 0 to 10 per cent in the course of six months, and the appearance of pathologic, "unripe" leukocytes together with proliferation in various tissues (Figs. 8 and 9). The experiment was then repeated on 8 spermophiles; 2 were double adrenalectomized, 3 were double-ovariectomized and 3 were used as normal controls. The results already described were found to repeat themselves both in blood and tissue.** The

*Observations to be published.
**Details will be reported in a future publication.

proliferation of the various types of tissues is comparable to the proliferation of the small arteries in lungs and kidneys together with changes in the spleen resembling those found in "Gaucher's spleen," which have been observed by McMeans after forced cholesterin feeding.

I have studied simultaneously in goats the effect of cholesterin feeding alone,

Fig. 8.—(Spermophile 28.) Proliferation of epithelial elements in the bladder of double adrenalectomized spermophile (citellus tridecemlineatus), which had been given light roentgen treatment (42 Holzknecht in the course of 6 months at distance of 24 inches from the tube). (H.E. × 300 diam.)

Fig. 9.—(Same as Fig. 8.) Proliferation in the outer zone of the kidney. (H.E. × 300 diam.)

the influence of the rœntgen rays alone, and the influence of cholesterin feeding combined with rœntgen treatment. Here also similar changes in the blood picture were observed. Six goats were used for this experiment.

The blood of our six goats was examined weekly for a period of six weeks before the experiments were begun. During this time the animals were kept

under uniform and constant conditions, and no great variations in their blood picture occurred. The most marked changes were those produced by cholesterin feeding alone.** These changes in one of the goats (No. 21) are shown in Table I.

Recently I have made a series of observations on the cholesterin content of the blood of patients suffering from malignant disease. The Autenrieth-Funk[45] method and Bloor's[46] modification thereof were used. In some cases Bloor's test gave slightly higher values, in others the results were identical. The highest value has been given in the table. The normal cholesterin value as given by Authenrieth and Bloor averages about 0.18 with a low limit of 0.14 per cent. The high normal value has not yet been determined, but I have found it as high as 0.27 per cent in apparently normal adults. Authenrieth has found a cholesterin value as high as 0.30 per cent in the blood of normal pregnant women. In a case of xanthoma tuberosum he reports a cholesterin value in the blood of 0.58 per cent before operation and 0.54 per cent after operation.

TABLE I.—CHANGES IN THE BLOOD-PICTURE OF GOAT FED CHOLESTERIN ONLY (GOAT 21).

(Results expressed in per cent.)

| Date 1915-1916. | Polymorphonuclear leukocytes. | Small lymphocytes. | Large lymphocytes. | Eosinophile leukocytes. | Mast-cells. | Transitionals.* | Neutrophile myelocytes | Normoblasts | Turk's "irritation cells." | Atypical small lymphocytes. | Tumor 1.** | Tumor 2.*** | Cholesterin. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Period I.—Control Period. | | | | | | | | | | | | | |
| Sept. 10 | 24 | 70.5 | 1.5 | — | 1.5 | — | 2 | — | — | — | — | — | — |
| Oct. 4 | 38 | 50 | 7.5 | — | 1 | 2 | 1.5 | — | — | — | — | — | 0.124 |
| 18 | 30 | 60.5 | 7.5 | 1 | 1 | — | — | — | — | — | — | — | 0.084 |
| 21 | 31 | 58.5 | 9.5 | — | — | — | 1 | — | — | — | — | — | 0.078 |
| 25 | 38 | 53.5 | 5.5 | — | 1 | 1 | 1 | — | — | — | — | — | 0.07 |
| 28 | 54.5 | 30.5 | 5.5 | 2 | 5 | 3 | — | — | — | — | — | — | 0.072 |
| Period 2.—0.30 Gm. Cholesterin Daily (Beginning Nov. 2). | | | | | | | | | | | | | |
| Nov. 8 | 28 | 25 | 25 | 10.5 | 4 | 6.5 | .5 | .5 | — | — | — | — | 0.15 |
| 15 | 27.5 | 12.5 | 10 | 24 | 10.5 | 2 | 1.5 | 1 | — | — | — | — | 0.186 |
| 22 | 26.5 | 14 | 10 | 15 | 7 | 2 | 4 | 3 | .5 | 6 | 3 | — | 0.146 |
| Dec. 6 | 26.5 | 9 | 10 | 5 | 5 | 1 | 7 | 10 | 1.5 | 15 | 5 | — | 0.158 |
| 13 | 44 | 2.5 | 6.5 | .5 | 8 | — | 11 | — | 1.5 | 20 | 3.5 | 1.5 | 0.158 |
| 20 | 33 | 8 | 24 | 2 | 1 | 4 | 11 | 4 | 2 | 12 | 9 | 2 | 0.14 |
| 27 | 30.5 | 9 | 13.5 | 3 | 3.5 | 2 | 8 | 2 | 1 | 18 | 9 | .5 | 0.186 |
| Jan. 5 | 25 | 8 | 19 | 2 | 7 | 6.5 | 10 | 2 | — | 18 | 2 | 1.5 | 0.168 |
| 10 | 30 | 5 | 12.5 | 3 | 10 | 4 | 12.5 | 4 | — | 9 | 9 | 1 | 0.158 |
| 17 | 19 | 5 | 11 | 7 | 11 | 16 | 14 | — | .5 | 15 | 8 | 1 | 0.14 |
| 24 | 27 | 30 | 11 | 5.5 | 4.5 | 11.5 | 2.5 | 2 | | | | | |
| | | | | | | | | 1 Meg. | — | 5.5 | .5 | — | 0.14 |
| Feb. 1 | 38 | 27 | 12 | 4 | 4.5 | 8.5 | 1 | — | — | 3.5 | — | 1 | 0.13 |
| 7 | 30 | 26.5 | 16 | 3 | 15 | 3.5 | 1.5 | 1.5 | — | 2.5 | — | .5 | 0.13 |

Hemoglobin, 20 per cent; erythrocytes, 16 million; leukocytes, 15,200; index, 0.7.

*Transitionals—variety of "large mononuclears," with kidney-shaped nucleus; the typical mononuclears with single round or slightly oval nucleus were not found in this count.

**Tumor 1.—term borrowed from Schleip's Atlas der Blutkrankheiten Leukosarcomatosis, p. 128, Fig. 61, with which the cells appeared to be identical.

***Tumor 2.—term borrowed from Schleip's Atlas (Carcinose des Knochenmarks), p. 124, Fig. 59, h., with which cells appeared to be identical.

The values found in the blood in the cases of malignancy that I examined are given in Table II.

Weltmann[47] has studied the cholesterin percentages found in the blood under various conditions of disease, and finds them relatively high in arteriosclerosis, nephritis, non-ulcerating tumors, disease of the liver, and diabetes, but as he uses another method (a modification of the Neumann-Hermann test with different scale and different color-reaction) and as he himself points out, "obtained not accurate but only comparative values," his data cannot be used for comparison with mine which are comparable only to Authenrieth's findings in the case of xanthoma tuberosum (0.58) referred to above.

As may be seen in Case 147020 (Table II), the patient having the highest cholesterin value of all showed not only symptoms of proliferation in the blood (109 normoblasts, 14 megaloblasts in a count of 300 cells; a picture typical of pernicious anemia according to Ehrlich; atypical according to Grawitz) but malignant degeneration in a number of histologically diverse tissues as well (Figs. 10 to 14).

In Case 140350 the formation of a rapidly growing, highly malignant tumor was preceded by a clinical history of unrecognized chronic appendicitis. The chronic inflammatory condition had lasted for several years and during this period the patient had gained considerably in weight. At operation uterine fibroids were found. After hysterectomy and unilateral ovariectomy, the men-

TABLE II.—CHOLESTERIN VALUES IN THE BLOOD OF PATIENTS WITH MALIGNANCY.

| Number. | Sex. | Age Years. | Civil State. | Diagnosis. | Treatment. | Cholesterin Per cent.* |
|---|---|---|---|---|---|---|
| 1- 147020 | F | 35 | M | General carcinomatosis with blood picture of pernicious anemia | Transfusions Autopsy | 0.710 |
| 2- 154152 | F | 55 | M | Epithelioma of tongue | Before treatment** | 0.502 |
| 3- 136512 | F | 39 | M | Pernicious anemia (?) | Two transfusions | 0.502 |
| 4- 152680 | F | 52 | M | Epithelioma of tongue | Before treatment | 0.474 |
| 5- 150864 | F | 49 | M | Mass in the sigmoid (benign?)+ | Before treatment | 0.446 |
| 6- 137213 | M | 45 | M | Recurrent cancer of lower jaw | Before treatment | 0.446 |
| 7- 150350 | F | 48 | M | Epithelioma of the arm; amputation++ (exarticulation at shoulder) | radium; treatments | 0.446 |
| 8- 151635 | M | 52 | M | Cancer (?) of the tongue, blastomycosis | Potassium iodide; iodine locally; radium | 0.266 |
| 9- 133093 | F | 65 | M | Multiple lymphomas | Operation; radium; Fowler's solution | 0.254 |

*Average of 10 to 12 readings on each specimen of blood examined.

**Radium appears to lower cholesterin values.

+Blood for cholesterin test was taken during anesthesia for operation; patient had been under the anesthetic for 15 minutes. The effect of ether-anesthesia on cholesterin values is being studied; the values seem to be lowered by anesthesia and by radium treatment.

++The sigmoid and colon descendens appeared to be involved though the character of the tumor could not be defined. Since the patient was in fairly poor health and did not present any clinical symptoms of malignancy, operation which would probably have meant the resection of two-thirds of the large intestine seemed contraindicated. The clinicians expressed the opinion, however, that operation might be necessary eventually on account of obstruction or the development of malignant symptoms.

strual period disappeared completely. A year later slight injury to a birthmark, which had never given any trouble (patient's age 47) resulted within two months in the development of a highly malignant tumor (Figs. 18 to 20). Five points in the history of this patient seem to deserve special attention : (1) a condition of chronic infection, which, existing during a considerable period, is likely to have overtaxed the adrenal, the chief organ of regulation in cholesterin metabolism,

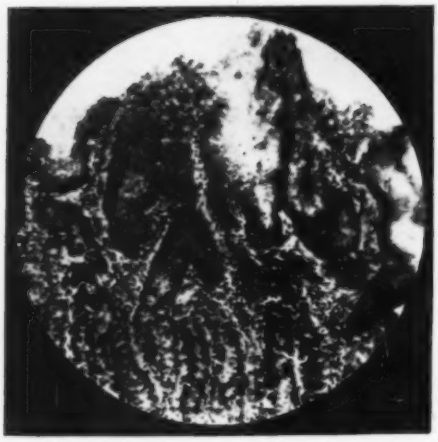

Fig. 10.—(147420.) Multiple primary tumors, atypical form of pernicious anemia, high cholesterin value in the blood (0.71%), malignant proliferation of the gastric mucosa. (H.E. × 250 diam.)

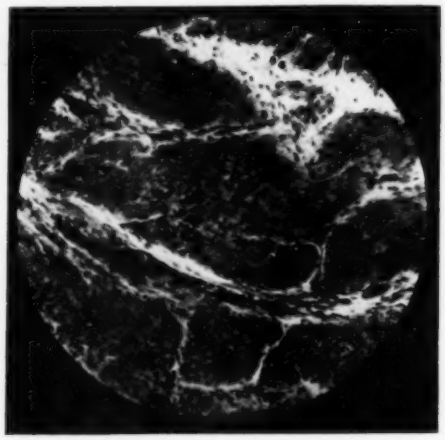

Fig. 12.—(Same as Fig. 10.) Invasion of the outer muscular strata. (H.E. × 600 diam.)

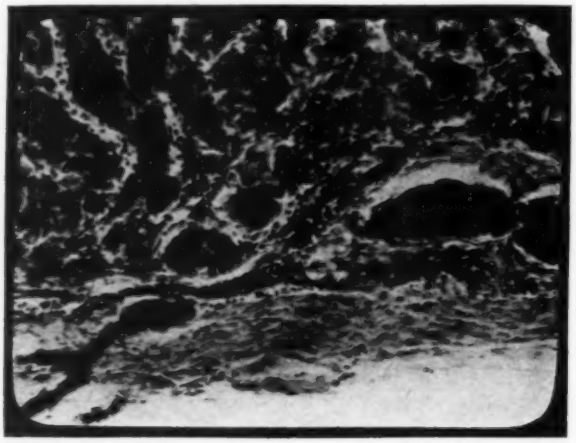

Fig. 11.—(Same as Fig. 10.) Malignant invasion of the muscularis mucosa. (H.E. × 350 diam.)

(2) the increase of body-fat observed in the patient, which must be considered a measure of defense against the accumulation of cholesterin, as has been stated by Rothschild, (3) the high cholesterin value of the blood, (4) the benign tumors found at operation which may be considered another evidence of existing hypercholesteremia, and (5) the highly malignant tumor which developed within a year after cessation of the menses and which may be said to suggest that reproductive activity and malignant growth are more closely related than has been universally believed.

The influence of the sex glands on cell proliferation has been studied by a number of writers (Graf, Almagie, Rohdenburg, Bullock, Johnson, Hilario[48]), the results of these investigations in many cases leading to conclusions diametrically opposed. Sweet, Corson-White and Saxon[49] found that "the receptivity for a tumor with moderate powers of proliferation is increased by castration and that the proliferative power of the neoplasm is augmented." The effect of

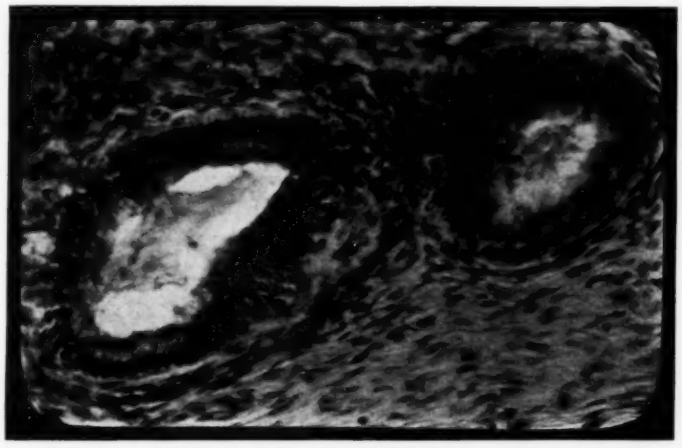

Fig. 13.—(Same as Fig. 10.) Malignant proliferation in the uterine glands (compare with Fig. 6). (H.E. × 600 diam.)

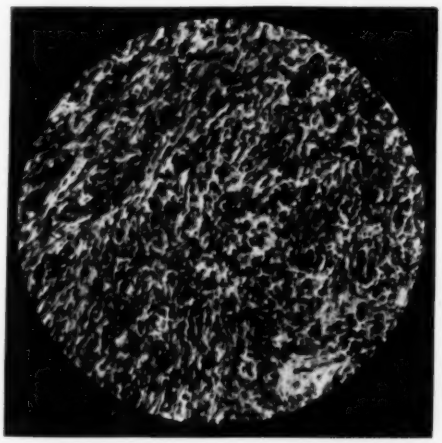

Fig. 14.—(Same as Fig. 10.) Left ovary, tumor 15 cm. diameter. Diffuse type of carcinoma. (H.E. × 350 diam.)

castration on the formation of benign tumors in the antlers of deer has been commented on by Lauterborn[50] and Tandler and Gross.[51]

Recently Lathrop and Loeb[52] have called attention to the influence of castration in young mice under six months by which the cancer incidence is decreased and the cancer age increased. They mention also "that non-breeding mice become fat at the age of 9 months, and at 15 months almost all are extremely fat —a change usually associated with a decline in the breeding activity." The fact that in these experiments, castration exercised a restraining influence on the

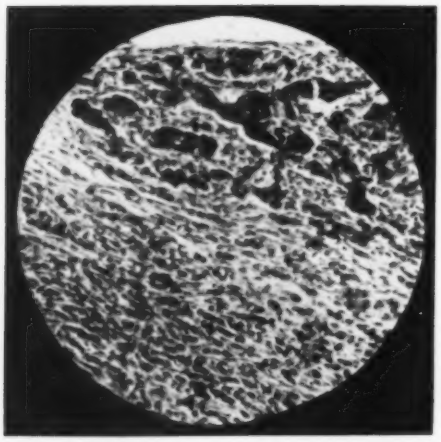

Fig. 15.—(Same as Fig. 10.) Tumor of right ovary, 6 cm. in diameter. Two types of carcinoma. Diffuse type mixed with solid strands. (H.E. × 350 diam.)

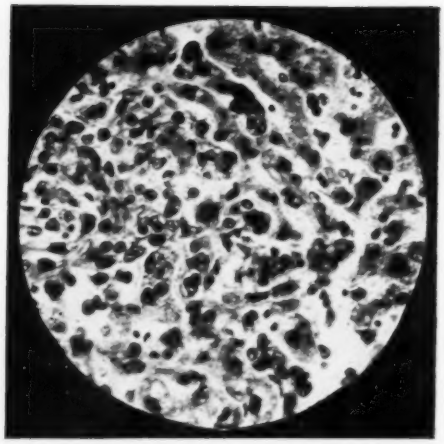

Fig. 16.—(Same as Fig. 10.) Malignant degeneration of the liver-"trabecula." This type of malignancy was observed in every section. No metastatic nodules were found. (H.E. × 300 diam.)

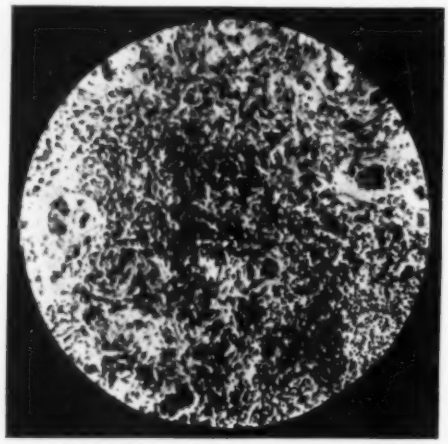

Fig. 17-*A*.

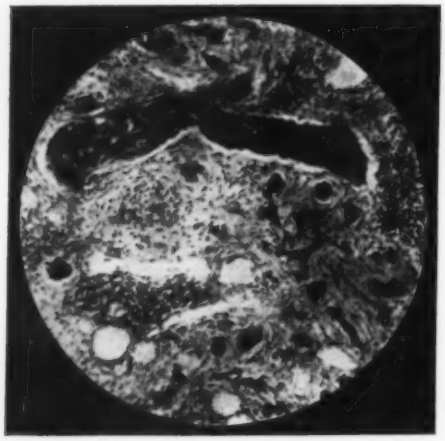

Fig. 17-*B*.

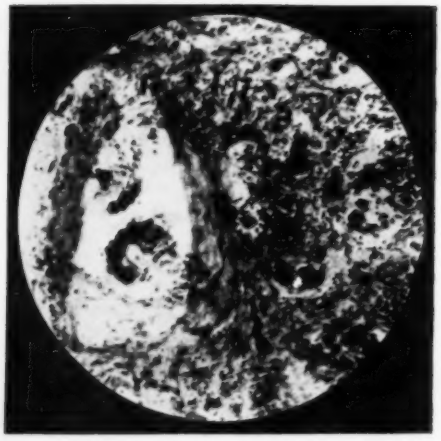

Fig. 17-*C*.

Fig. 17, *A*, *B*, *C*.—(Same as Fig. 10.) Lumbar lymphatic gland containing three different types of carcinoma of metastatic origin (*A*, diffuse; *B*, solid strand; *C*, adenoma). (H.E. × 250 diam.)

progress of malignancy only when the operation was performed on young animals seems to suggest that the young organism, not yet entirely grown, might be able to utilize any surplus of cholesterin resulting from castration for purposes of natural growth, or that it has greater powers of elimination at its command. I was able to observe a distinct increase of cholesterin in the blood of goats following castration and the same observation has been made by Lowenthal[58] in rabbits. The increase of body fat usually observed in castrates and noted by

Fig. 18.—(150350.) Melano-epithelioma originating from a birthmark after slight injury.

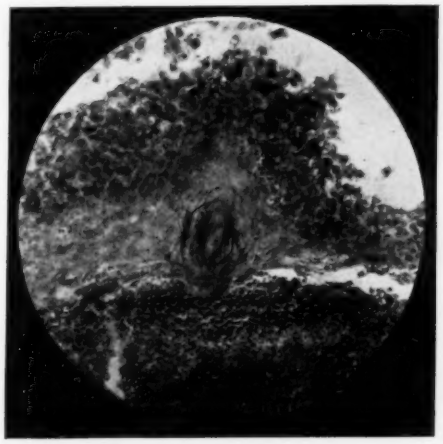

Fig. 19.—(Same as Fig. 18.) Carcinomatous portion of the tumor. (H.E. × 350 diam.)

Lathrop and Loeb in their non-breeding mice would seem to indicate (in connection with other evidence concerning the physiologic activity of cholesterin) that the generative organs utilize a certain proportion of the available cholesterin for the formation of the reproductive cells.

## SUMMARY.

1. Clinical data and experimental observation alike suggest that the causative factor in the production of malignant proliferation is widely distributed within the organism.

2. There is reason to believe that this fundamental factor is chemical in its nature.

3. The existence of a gradual transition between normal, reparative, benign and malignant proliferation has been commented on by many observers.

4. The evident connection observed between an increase of cholesterin and proliferation, both normal and abnormal, seems to suggest that cholesterin may act as a stimulant to cell-division.

5. After puberty and under normal conditions the process of cell-division is constantly demonstrable in the sex glands.

6. Recent investigations have shown that the sex glands appear to take an active (perhaps a prominent) part in the regulation of cholesterin metabolism.

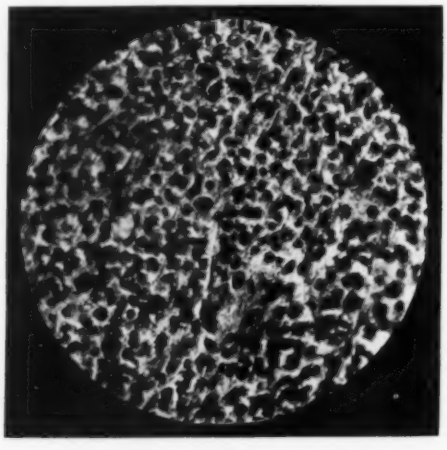

Fig. 20.—(Same as Fig. 18.) Sarcomatous portion of the tumor. (H.E. × 800 diam.)

As cholesterin is stored in considerable quantities in the corpus luteum during pregnancy, and increases in the blood after castration, it seems permissible to assume that it may in some way stimulate the formation of the generative cells.

## CONCLUSION.

These observations, the fact that the cancer age coincides with the cessation of reproductive activity as shown by the cancer statistics (Prinzing[54]), the importance of metabolism emphasized by Hoffmann in his statistical review on the cancer rates of the Western Hemisphere, and the influence of heredity (the transmission of inadequate organs) established by the work of Slye,[55] all seem to support the view that the retention of cholesterin due to its insufficient conversion or defective elimination may be a primary factor in the etiology of malignant disease.

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# THE PRESENT STATUS OF TUBERCULOSIS CHEMOTHERAPY*

By Lydia M. DeWitt, Chicago, Ill.

TUBERCULOSIS is a disease of so irregular a course, with such a tendency to periods of latency or intermission, alternating with periods of acute exacerbation and long periods of slow, chronic progress, that it is most deceptive in its therapy. Any treatment begun just before an intermission seems to be the cause of the apparent improvement, while one begun before an exacerbation is regarded as injurious. These facts make a judgment based on clinical evidence unreliable unless it extends over long periods and includes a large number of patients. Among experimental animals, moreover, we have some, like the guinea-pig, so extremely susceptible that they seem to have no natural forces of resistance and the disease is almost uniformly fatal regardless of treatment. If anything would cure the disease in guinea-pigs, therefore, it would almost certainly do so in man, who is much less susceptible. But it is quite possible that some treatment might cure the disease in man which would have little or no effect in the guinea-pig. The rabbit and the mouse, on the other hand, are much more resistant to human tuberculosis than is the guinea-pig, but the pathologic character of the disease produced by infection is quite unlike human tuberculosis. Hence no experimental animal has yet been found in which conditions are quite comparable to those in man. It is necessary, therefore, to use the available experimental animals in testing toxicity, determining dosage and palliative, if not curative effect; in other words in "getting a lead" as to the usefulness of any proposed method of treatment. If we find that, in a large series of animals as susceptible as the guinea-pig, a given treatment uniformly and consistently delays death, lessens the dissemination of the tubercles, increases connective tissue formation and diminishes the virulence and virility of the tubercle bacillus, even though the infection is not cured, we have a right to conclude that it is worthy of consideration and of trial on human patients. Even then it must be subjected to long periods of trial on large numbers of patients before it can be accepted as of value in tuberculosis therapy.

Since the failure of tuberculin in all its forms as a specific cure for the disease, investigators have turned almost with one accord to chemotherapy, believing that in the vast number of chemical substances and the endless variety of possible combinations of these, some might be found having a specific beneficial action on tuberculosis. These investigators have been encouraged by the success of Ehrlich and his coworkers in protozoan and spirochetal infections. In tuberculosis, however, we have quite a different problem. Instead of a delicate, naked organism circulating in the blood stream, we have an organism protected by a difficultly permeable membrane and rarely found in the circulating blood, but usually in nonvascular and often necrotic cells or groups of cells. These necrotic masses are readily permeated by crystalloid and semicrystalloid substances, which influence the tubercle favorably or unfavorably, first by break-

*From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the University of Chicago.

ing down the tissue and permitting its absorption, second by favoring connective-tissue formation and calcification (Nature's method of effecting a cure); third, by attacking the bacilli themselves, whether intra- or extracellular. Certain true colloids also may be taken up by phagocytic cells and carried to the tubercles. The principal methods of attack are: (a) direct, on the organism of the disease, killing it or inhibiting its growth; and (b) indirect, on the protective agencies of the body.

Relatively few chemical compounds have as yet been tested with relation to their value in tuberculosis and it is the purpose of this paper to give briefly the present status of chemotherapy in tuberculosis.

## IODINE.

Iodine is one of the substances longest used in the treatment of tuberculous patients. Its earlier use was no doubt purely empirical. In 1905, however, Cantacuzène,[1] as the result of an experimental study, reported that fat-free tubercle bacilli lose much of their toxicity if treated with an iodine solution previous to their injection and that they are then absorbed much more readily than the noniodized bacilli. He also states that fat-free bacilli and tubercles formed from them in guinea-pigs are much more rapidly absorbed if potassium iodide is administered to the animals daily, the iodine salt stimulating the phagocytic power of the endothelial cells. Hirsch[2] repeated these experiments and enlarged upon them, but was unable to verify the results obtained by Cantacuzène. Hirsch concludes that there is no experimental proof that iodine and iodides facilitate the absorption of necrotic tissue and organization of tuberculous and other granulation tissues. The daily administration of potassium iodide does not hasten the removal of tubercle bacilli by stimulating the phagocytic power of endothelial cells. Iodized fat-free tubercle bacilli are absorbed no faster than are the noniodized. Loeb and Michaud[3] found that, if iodine in various forms was injected into tuberculous animals, much more iodine was found in tuberculous than in normal tissues. This finding was confirmed by Wells and Hedenburg,[4] who, however, found that this property was not specific for tuberculous tissues, but was common to other necrotic tissues and due to the fact that dead and injured cells are more easily permeated by crystalloids. Lewis[5] also found a greater amount of iodine in tuberculous than in normal tissues of animals treated with iodine compounds, but he also found more iodine in tuberculous than in normal tissues of animals not intentionally treated with iodine. No systematic experimental work has as yet been published on the therapeutic action of iodine in tuberculosis. DeWitt and Sherman[6] found that Lugol's solution, full strength, could not be depended upon to kill tubercle bacilli in twenty-four hours and I have as yet found little, if any, therapeutic effect from the use of various iodine dye compounds used by me in the treatment of tuberculous guinea-pigs, although von Linden claims considerable value for her iodine compound of methylene blue.

## ARSENIC.

Arsenic also is one of the drugs long used more or less empirically in the treatment of tuberculosis and it is natural that, after the success attending its use in certain spirochete infections, the attention of investigators on the chemo-

therapy of tuberculosis should be turned to arsenic and its compounds. In 1914, Schumacher[7] published a communication on salvarsan as a true dye, in which he points out that arsenic destroys many bacteria and other disease agents to which it is able to gain access, but ascribes its slight effect on the tubercle and related organisms to the fact that their waxy envelope prevents penetration and thus protects them from the toxic influence of the arsenic. He suggests that salvarsan might be made more effective by combining it with some substance which could injure the tubercle bacillus in the body, such as cholin. He also suggests combining it with gold or gold-containing dye, so constituted that colloidal gold could be split off. Charpentier[8] finds that tubercle bacilli grow well in the presence of sodium arsenate or of atoxyl; not quite so well in methyl-arsenate of sodium or cacodylate of sodium. Chemical tests show arsenic in the bacillary masses grown on media containing arsenic; he also finds arsenic in media to which he has added no arsenic, a fact which he explains by the presence of arsenic in most animal tissues and hence in protein containing media. He also finds that sodium cacodylate in no way modifies the progress of a tuberculous infection in guinea-pigs inoculated with virulent cultures and ascribes its favorable influence in human tuberculosis to its power to increase the natural forces of resistance which are present in man but not in the guinea-pig. In 1914, in a communication by Wells, DeWitt and Corper[9] a reference was made to work being carried on by Corper and Arkin on the action of arsenic in experimental tuberculosis. The complete report of their work has just appeared in the *Journal of Infectious Diseases*.[33] They used sodium arsenate, sodium cacodylate, atoxyl, arsacetin and neosalvarsan. These preparations do not kill the tubercle bacillus *in vitro;* they penetrate tuberculous tissues, but not specifically; they have no effect on the progress of the disease in animals.

### SODIUM SULPHOCYANATE.

Sodium sulphocyanate was also tested by Corper[10] and found to have no chemical affinity for tuberculous tissues and no bactericidal effect on tubercle bacilli *in vitro*.

### DYES—VITAL STAINS.

Dyes which can be introduced into the living body without material injury have for several years interested therapeutists in several fields. Thus Ehrlich began his famous series of experiments on the chemotherapy of spirochete infections with the dye trypan red, which was itself trypanocidal *in vivo*. Wassermann used eosin as a carrier for selenium in his cancer experiments. Ehrlich found methylene blue of some value in the treatment of neuralgia and malaria, and Einhorn used it as early as 1891 in the treatment of pulmonary tuberculosis. Lewis is experimenting with compounds of trypan red and trypan blue in the treatment of tuberculosis, and Weil[11] has recently published a communication on the use of certain dyes and dye compounds in the study and treatment of rat tumors. Congo red and some of its compounds were used without therapeutic effect, though they penetrated the tumors.

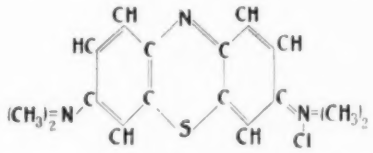
With my assistants and coworkers, I[12] have for some years been investigating numerous dyes and dye compounds with relation to experimental tuberculosis. In this study, we have endeavored to ascertain the power of the va-

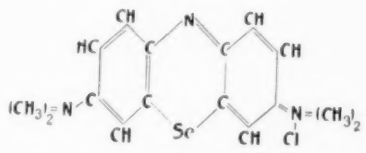
rious dyes to penetrate and stain the tubercle and the individual tubercle bacilli; their ability to kill the tubercle bacillus or inhibit its growth; their effect on the development of experimental tuberculosis in animals. Because both tubercle bacilli and tubercles are rich in fats, it seemed reasonable to think that fat soluble dyes might be more efficient than fat insoluble ones. Sherman,[14] however, found that the ordinary fat dyes, sudan iii, sudan yellow and sudan brown, scarlet R, nile blue sulphate, etc., penetrate and stain the individual tubercle bacilli but little, if at all, although clumps and masses of culture show a mass stain. Corper[13] also reported that these dyes, when administered to tuberculous guinea-pigs until the normal body fats were brilliantly stained, were never found within the tubercle and never stained the bacilli within the tubercles. The course of the tuberculous infection was never influenced favorably by the administration of these dyes, whether given in water or oil solutions, by injection or by mouth. As to dyes not soluble in fats, it was found by Goldmann[15] and also by Lewis[16] that the trypan dyes penetrated to the center of tubercles. I also ascertained that trypan blue easily penetrated and was retained in tubercles, whether young and epitheloid in character or having necrotic and caseous centers, and also into the large necrotic areas so common in the liver and spleen of tuberculous guinea-pigs. These dyes are found in certain phagocytic endothelial cells in the form of irregular, deeply stained, difficultly soluble granules, while in the caseous centers the dye is partly homogeneous and partly finely granular. The tubercle bacillus is stained but faintly, if at all, and is neither killed by the dyes nor inhibited in its growth. Not even is its virulence diminished, as animals infected with cultures which had been exposed to these dyes and inoculated animals treated with these dyes, develop the disease as rapidly and die as quickly of a severe generalized infection as do the untreated control animals. Lewis[17] has also used trypan red compounded with iodine, thymus, eucalyptol, guaiacol and iodoform, with no appreciable influence in prolonging the course of the infection, although these compounds resemble the original dye in their power to penetrate the tubercle. DeWitt, also, in the hope of increasing its therapeutic power, compounded trypan blue with copper, silver, iron, and mercury. These salts were, however, more toxic and had little more influence on the course of the disease than the trypan blue, if we except the mercury compounds. The animals treated with these latter lived longer than the others and had very slight tuberculous involvement, either local or general.

I also found that all the various methylene blues tested penetrated the tubercle and stained the tubercle bacilli *in vitro* very well; that basic fuchsin, crystal violet, erythrosyn and the eosins also stain the tubercle bacillus well; and that methylene blue, bismarck brown and brilliant cresyl blue have some bactericidal power and a marked inhibitory influence on the growth of the tubercle bacillus *in vitro*. The purified methylene blue of the pharmacopeia has but little general toxicity, though subcutaneous injections cause infiltration and ulcerations. Though methylene blue possesses so many qualities which should theoretically make it valuable as a therapeutic agent in tuberculosis, my experiments have not yet shown any considerable curative effect in the experimental disease in guinea-pigs. Von Linden reported rather better results than mine,

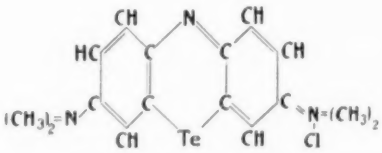
especially with the use of an iodine compound, the method of making which and the formula for which she does not divulge. She states that this dye prolonged life materially, caused healing of the initial sinus and abscess, increase of weight, lowering of fever and in one case perfect healing and sterilization.

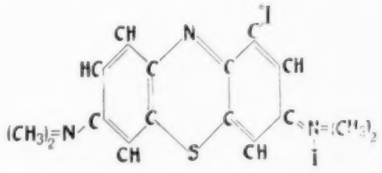
Some modifications of methylene blue have been made for me. In one case, the sulphur of the methylene blue molecule was replaced by selenium and





in another by tellurium. These modifications were suggested to me by the finding of Gosio[18] and Belfanti[19] that the salts of selenium and tellurium have a pronounced bacteriotropic power over the tubercle bacillus and could perhaps be used as the point of origin for new therapeutic preparations. My selenium and tellurium blues were, however, weaker, less stable, and more toxic dyes





than methylene blue and showed no advantages over it. An iod-methylene blue has also been made for me, in which one iodine atom is in the ring. My experiments with this are still under way and will be reported in detail later.

HEAVY METALS.

In 1914, DeWitt and Sherman[20] published the results of a series of investigations on the bactericidal action of numerous chemical disinfectants on the human tubercle bacillus. Growth tests were made on artificial media and life and virulence tests on guinea-pigs. It was ascertained that 0.001 per cent of mercuric chloride killed within twenty-four hours all organisms, if spread out in a thin layer on glass beads. It required 0.004 per cent to destroy their power of growth in twenty-four hours, if the organisms were clumped. Gold chloride came next in bactericidal power, 0.005 per cent killing within twenty-four hours all tubercle bacilli, if spread out in a thin layer on glass beads. If in clumps, it required 0.01 per cent to destroy their power of growth. It was stated by Koch[21] that one part of gold cyanide in 2,000,000 parts of media completely inhibited the growth of tubercle bacilli, a statement which I have been able to verify. Silver nitrate is slightly less bactericidal for these organisms, 0.01 per cent killing them within twenty-four hours, if in thin layer and 0.05 per cent, if in clumps.

Copper chloride stands lowest of all the metals tested, as it required 5 per cent to kill in twenty-four hours all the bacilli, if spread out in a thin layer and even 25 per cent did not kill all if they were in clumps. Von Linden claimed

that one part of copper chloride in 1,000,000 parts of media inhibited the growth of tubercle bacilli and that one part in 100,000 killed all, even in clumps. Feldt[22] found that for inhibition one part in 5,000 up to one part in 50,000 was needed according to the preparation used. In our own work we found it necessary to use at least one part of copper chloride in 100,000 parts of media to completely inhibit development of the culture. In spite of its low tuberculocidal power, copper has received more attention than the other metals from investigators in the chemotherapy of tuberculosis. As early as 1885, Luton[23] recommended the use of copper in human tuberculosis and to some extent it has been used ever since. It came into renewed prominence in 1912, when Gräfin v. Linden reported before the Tenth International Congress for Tuberculosis that, if treatment of guinea-pigs with copper and methylene blue was begun within fourteen days after inoculation with a tubercle culture of relatively low virulence and continued for six months, the disease was checked or healed in 75 per cent of the cases. The copper was more energetic in its action than the methylene blue. Since 1912, v. Linden[24] has published numerous preliminary communications and in 1915, a complete and detailed report of her work. Many other investigators have attempted to verify her work and have failed to obtain any results comparable to hers, while every claim put forward by her has been contradicted by careful investigators. Moewes and Jauer[25] used several compounds of copper, among them the preparations recommended by v. Linden and found them uniformly toxic for animals and useless, so far as influencing the progress of the disease is concerned. Corper[26] analyzed for copper the tissues of many tuberculous animals treated for long periods with different preparations of copper and found no appreciable amount of copper in the tuberculous areas and observed no effect on the course of the disease.

GOLD.

Basing their experiments on the previously quoted statement of Koch regarding the high inhibitory value of gold salts, reports of experiments on the therapeutic power of gold salts in tuberculosis were published in 1913 and the following years by Bruck and Glück, by Spiess and Feldt, by Mayer[30] and others. The work of Bruck and Glück[27] is concerned mainly with the action of gold potassium cyanide, with which they obtained good results, especially in lupus. Feldt[28] and Spiess and Feldt[29] have made and patented under the trade name "Aurocantan" a compound of gold cyanide with ethylene-diamine-cantharidin. The compound used is mono-cantharidin-ethylene-diamine-aurous cyanide. They state that both cantharidin and gold are specific for tuberculosis, that the toxicity of cantharidin is greatly reduced by condensation with ethylene diamine, and that the aurocantan is much less toxic and more effective than the gold cyanide alone. The preparation is administered intravenously and has been used alone and also combined with other treatment; e.g., tuberculin, borcholin, and with ultra violet rays. They consider that the effect of the gold is due largely to its oxygen carrying power. Its effect in tuberculous animals consists, according to the claims of these workers, in increase of weight, prolongation of life, checking the spread of the disease in the internal organs and favoring connective tissue encapsulation of the tubercles. They also claim

that the tubercle bacilli in the body become thin, granular and strongly agglutinated. In patients with open pulmonary tuberculosis, the sputum diminished in amount, the tubercle bacilli disappeared, the amount of sputum protein diminished and physical signs improved. These workers say that, as silver is specific for pyogenic cocci, arsenic for spirochetes and copper for algæ, so is gold specific for tubercle bacilli. I have for some months been carrying on investigations with gold cyanide and gold potassium cyanide in the treatment of experimental tuberculosis in animals, but my work has not yet progressed far enough for me to be willing to either affirm or deny the claims of these investigators.

MERCURY.

So far as I have been able to ascertain, no work on the mercury therapy of experimental tuberculosis has as yet been published, in spite of the fact that this metal stands at the head of the heavy metals in its tuberculocidal power. The high toxicity of the simpler mercury salts has necessitated the making of new preparations less toxic for the animal tissues while still preserving a high parasiticidal and inhibitory power. Many complex organic compounds have been made and tested as to toxicity, distribution and influence on diseases caused by spirochetes and spirilla. Among these, mention may be made especially of numerous so-called mercurialized dyes, made and tested by several investigators, notably Hahn and Kostenbader.[31] In one group of these dyes, the mercury is bound to nitrogen, oxygen, or some group outside of the ring; in a second group, the mercury is attached by one valence to a carbon atom in the ring, the other valence being joined to oxygen or some other atom or group; in the third group, the mercury connects two rings, both of its valences being bound to carbon. Some of these compounds were made synthetically, others by substitution in the dye molecule. Jacobs and Heidelberger[32] have made a number of mercuric acetate derivatives of the aromatic amines, most of their compounds having a dye character. For some months, I have been carrying on investigations on the action of some mercury dye compounds, as well as other mercury salts. My experiments are still in progress and it is too early to give any decisive answer to the question whether mercury can be considered efficient in the treatment of tuberculosis. Most of the preparations used are toxic and have had no apparent beneficial influence on the course of the disease. A few, like mercury nucleide and 1-amino-2(p-naphthalene-azo-phenyl mercuric acetate)5 sulphonic acid have consistently diminished the dissemination of the tuberculous lesions. Tuberculous animals treated with mercurialized methylene blue have lived much longer than untreated controls and the distribution of tubercles in the internal organs has been much less extensive. A complete and detailed report of all these investigations will be published later.

So far, then, our chemotherapeutic studies have resulted in no experimental demonstration of any chemical specifics for tuberculosis. The evidence regarding iodine, arsenic, copper and most of the dyes has been in the main negative. Gold, mercury and certain dye compounds are still on trial and no decisive results have yet been obtained. It must be concluded, therefore, that we are still just at the beginning of the experimental study on the chemotherapy of tuberculosis.

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# SUGAR CONTENT OF THE CEREBROSPINAL FLUID IN HEALTH AND DISEASE.*

By Walter M. Kraus, A.M., M.D., and G. G. Corneille, M.D.,
New York City, N. Y.

THE clinical value of the estimation of the sugar content of the cerebrospinal fluid is still open to discussion. A series of cases were therefore tested with the idea of determining the changes which disease, particularly syphilis of the nervous system, might cause.

In the natural course of events, many cases which were suspected to be syphilitic did not show any evidence of this by biological tests. These cases were, as far as the central nervous system was concerned, normal. The investigations of von Jaksch[1] in twenty cases showed the range of normal to be from .06 to .08 per cent. Kopetsky[2] used Benedict's copper method and found an average value of .046 per cent in eight cases. Mestrezat[3] found the average in twenty cases to lie between .048 and .053.†

*Method.*—The spinal fluid was examined within a few minutes of withdrawal. The globulin was tested and spinal fluid count and Wassermann were done. Sugar was determined by the Lewis and Benedict[4] method.

*Observation.*—Determinations were made fifty times in forty-six cases. These fall into three general groups.

(1) Normal. Table I. These cases were so considered because there was no evidence of organic involvement of the nervous system.

(2) Syphilitic disease of the brain, spinal cord and meninges. Table II.

(3) Purulent meningitis. Table III.

The average of normal cases was .0818 per cent, the range .055 to .110 per cent. The average for syphilitic cases was .0815 per cent, the range .055 to .110 per cent. There was no correspondence between the amount of globulin, the number of cells and the sugar content. Syphilis apparently does not alter the normal sugar content.

Two cases of purulent meningitis were followed daily.

Table I. Normal Cases.

| NO. | DIAGNOSIS. | % SUGAR. | WASSERMANN'S BLOOD | WASSERMANN'S SP. FL.‡ | CELLS. | GLOBULIN. | REMARKS. |
|---|---|---|---|---|---|---|---|
| 3 | Normal | .060 | 0 | 0 | 0 | 0 | |
| 22 | " | .072 | 0 | 0 | 0 | 0 | |
| 39 | " | .100 | 0 | 0 | 0 | 0 | |
| 57 | " | .090 | 0 | 0 | 0 | 0 | |
| 56 | Chr. Card. Valv. Dis. | .100 | 2 | 0 | 0 | 0 | |
| 19 | " | .086 | 0 | 0 | 0 | 0 | |
| 41 | " | .100 | 0 | 0 | 0 | 0 | |
| 7 | Unclassified Psychopathy | .074 | 3 | 5 | 0 | 0 | |

*From the Fourth Medical Division and the Psychopathic Department of Bellevue Hospital, New York.

†Read before the Neurological Section of the New York Academy of Medicine, Feb. 8, 1916. Just prior to the reading of this paper, one appeared in The American Journal of the Medical Sciences, by A. H. Hopkins (Vol. cl, No. 6). According to his observations, the normal lies between .06 per cent and .075 per cent. The Bang method was used.

‡=18 units is equivalent to a completely positive reaction.

TABLE I.—NORMAL CASES (CONT'D).

| NO. | DIAGNOSIS. | % SUGAR. | WASSERMANN'S BLOOD SP. FL. | | CELLS. | GLOBULIN. | REMARKS. |
|---|---|---|---|---|---|---|---|
| 4 | Manic Depressive Insanity | .045 | 0 | 0 | 1 | 0 | |
| 27 | | .110 | 0 | 0 | 1 | 0 | |
| 48 | | .088 | 0 | 0 | 0 | 0 | |
| 21 | Allied to Dementia Precox. | .110 | 0 | 0 | 3 | trace | |
| 28 | | .104 | 0 | 0 | 1 | 0 | |
| 52 | Dementia Precox | .100 | 0 | 0 | 0 | 0 | |
| 49 | Paranoid State | .095 | 6 | 0 | 0 | 0 | |
| 54 | Chronic Alcoholism | .110 | 0 | 0 | 2 | 0 | |
| 25 | Acute Enteritis | .070 | 2 | 0 | 0 | 0 | |
| 14 | Gas Poisoning | .110 | ? | 0 | 3 | 0 | |
| 39 | Abdominal Tumor | .080 | ? | ? | 2 | 0 | |
| 16 | Infective Exhaustive Psychosis | .073 | 15 | 0 | 0 | 0 | |
| 10 | Gen'l Arteriosclerosis | .065 | 4 | 2 | 1 | 0 | |
| 5 | " | .066 | 0 | 0 | 0 | 0 | |

TABLE II. SYPHILIS OF THE CENTRAL NERVOUS SYSTEM.

| NO. | DIAGNOSIS. | % SUGAR. | WASSERMANN'S BLOOD SP. FL.‡ | | CELLS. | GLOBULIN. | REMARKS. |
|---|---|---|---|---|---|---|---|
| 6 | Gen'l Paralysis | .098 | 15 | 18 | 10 | ++ | |
| 8 | " | .110 | 16 | 18 | 14 | ++ | |
| 9 | " | .075 | 18 | 18 | 31 | ++ | |
| 23 | " | .071 | 15 | 15 | 11 | ++ | |
| 29 | " | .072 | 15 | 18 | 72 | ++ | |
| 30 | " | .060 | 10 | 15 | 25 | ++ | |
| 38 | " | .068 | 12 | 15 | 21 | ++ | |
| 12 | " | .085 | 18 | AC | 62 | ++ | |
| 53 | " | .090 | 0 | 0 | 1 | 0 | |
| 31 | " | .072 | 15 | 15 | 12 | + | |
| 50 | " | .090 | 14 | 15 | 44 | + | |
| 42 | " (Juvenile) | 090 | 15 | 15 | 10 | + | Average Gen'l Paralysis=80 |
| 37 | Tabes Dorsalis | .055 | 9 | 15 | 198 | ++ | |
| 39 | " | .080 | ? | ? | 39 | + | |
| 43 | " | .090 | ? | ? | 39 | + | Average Tabes Dorsalis=75 |
| 17 | Cerebrospinal Sypnilis | .068 | 14 | 14 | 20 | ++ | |
| 40 | " | .020 | — | 15 | 8 | + | |
| 36 | " | .110 | 6 | 18 | Contaminated | Contaminated | |
| 34 | " | .060 | 17 | 18 | 157 | + | |
| 13 | " | .082 | 16 | 5 | 2 | + | |
| 20 | " | .075 | 15 | 15 | 11 | + | Average Cerebrospinal Syphilis=86 |

‡=18 units is equivalent to a completely positive reaction.
Ac=Anticomplementary.

TABLE III. PURULENT MENINGITIS.

| NO. | DIAGNOSIS. | % SUGAR. | CELLS. | GLOBULIN. | REMARKS. |
|---|---|---|---|---|---|
| *Case 1.* | | | | | |
| 2/19 | Meningococcus | .045 | 12,000* | ++++ | |
| 2/20 | " | .050 | 6,500** | +++ | |
| 2/22 | " | .055 | 188 | ++ | |
| 15/23 | " | .068 | 99 | ++ | |
| 24/24 | " | .080 | ? | ? | Recovered |
| *Case 2.* | | | | | |
| 12 | Pneumococcus Meningitis | .022 | 1,300 | ++ | |
| 13 | " | .015 | 9,000 | +++ | Died |

*=87% polymorphonuclears.
**=85% polymorphonuclears.

It will be seen from Table 3 that in the case which recovered the value steadily rose, while in that which died it fell.

The observation that the reduction of Fehling's solution (or Benedict's) is absent, i.e., that sugar is absent in acute purulent meningitis, has not been found to be true, a fact emphasized by Du Bois and Neal,[5] and Schloss and Schroeder.[6]

*The change in the amount of sugar in purulent meningitis is of great value from a prognostic point of view. A rise is favorable and a fall unfavorable.* This is emphasized by Hopkins and by a number of others quoted by him as well as by Schloss and Schroeder. The determination in syphilis of the nervous system adds nothing of value.

We wish to express our thanks to Dr. Cyrus J. Strong, Dr. Alexander Lambert and Dr. M. S. Gregory for permission to use cases on their services.

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# AN ARTIFICIAL RESPIRATION APPARATUS FOR GENERAL LABORATORY PURPOSES *

By Paul J. Hanzlik, M.D., Cleveland, Ohio.

THERE are many kinds of simple and elaborate apparatuses for securing artificial respiration. It would seem, therefore, superfluous and unnecessary to add to the list. However, as a rule, these are not suitable for the use of large classes of students. Many are intended for a single animal, or at most two or three. If suitable for more than one animal the rate and volume of respiration cannot be altered for any particular animal, since the mechanism provides for no individual variation. This is inconvenient and undesirable, especially in teaching in laboratories where animals in any lot may differ very widely in size, and the respiratory requirements for different experiments may differ considerably.

For these and other reasons, it seemed desirable to devise an apparatus which would obviate these difficulties; and so as to be generally serviceable for both teaching and research. At the same time, such an apparatus should be simple, and reasonable in price. It is believed that these requirements have been met to a large extent by an apparatus which has been devised and is now in successful operation in this laboratory.

The apparatus is built on a unit plan system, and consists briefly of a long, motor-driven shaft which in turn drives a series of pumps by a set of pulleys which permit the regulation of the rate and volume of respiration. Each pump and fittings properly attached to a table constitute a unit, and these may be multiplied up to the driving capacity of the motor. Each table is detachable, the apparatus is practically out of view and can occupy what might be termed the "dead-space" in the laboratory, that is, the main shaft is attached to the floor alongside the wall, and the pumps are situated underneath the table tops. Fig. 1 illustrates all of the attachments necessary in the makeup of a unit, with the exception of the motor. Figs. 2 and 3 illustrate in detail the parts used in the construction of several portions of the apparatus. These and other parts may now be described.

*Motor.*—The size will be determined by the number of units it is desired to put into operation. The exact horsepower to operate a single unit has not been determined, but a 1/16 Hp. motor will operate two units, and we have five units now in operation by a 1/4 Hp. motor. It is safe to assume that a 1/4 Hp. motor can easily operate 8 to 10 units. A worm attached to the motor shaft operates a cog-wheel on the main shaft, which rotates 26 times per minute. The speed of the motor was reduced by a specially cast cog-wheel. The motor had 700 r.p.m. In order to obtain 26 r.p.m., on the main shaft, a cog-wheel with 24 teeth was cast. (Theoretically, this wheel should give 29.1 r.p.m.) In order to reduce friction and noise as much as possible, the gears are immersed in an oil bath. It may be mentioned here that the motor is the most expensive part of the apparatus. However, this can be considerably mitigated by securing a used motor, which for ordinary laboratory purposes will serve very well.

*Main Shaft.*—This consists of an iron rod 15/16 inch in diameter, running through hangers, screwed to the floor close to the wall. A 30-foot length is advisable for 5 units. This secures plenty of working space between tables.

*From the Pharmacological Laboratory, Western Reserve University, Cleveland.

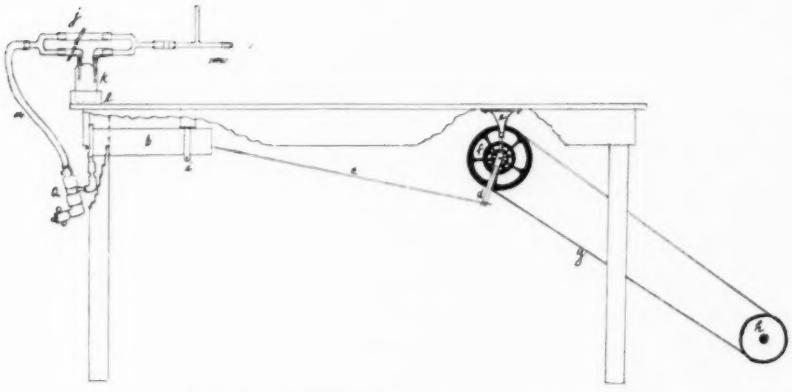

Fig. 1.—Artificial respiration unit (sectional view, scale about 1 to 20).

*a.* valve.
*b.* cylinder (pump).
*c.* piston shaft.
*d.* slit crank.
*e.* adjustable hanger.
*f.* iron grooved pulleys.
*g.* round belt.
*h.* wooden grooved pulley (with cross section of main shaft in center).
*i.* brass collar for holding pump.
*j.* anesthetizing attachment (Y-pieces placed upright; natural position horizontal).
*k.* Woulff bottle.
*l.* drilled wooden block
*m.* tracheal T-cannula.
*n.* rubber tubing connection.

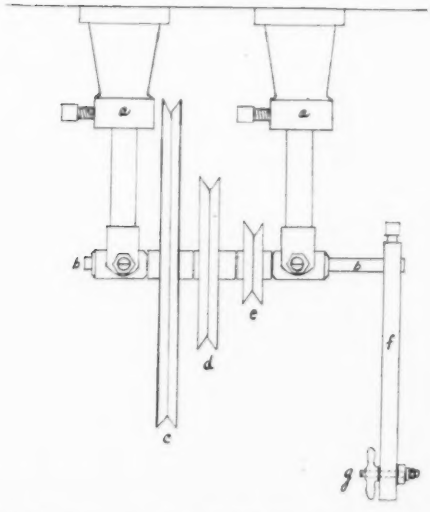

Fig. 2.—Adjustable hangers with iron grooved pulleys and slit crank (front view, scale about 1 to 4.6).

*a.* adjustable hanger (5½ inch).
*b.* iron shaft (½x8 inches).
*c.* 8-inch iron grooved pulley.
*d.* 4-inch iron grooved pulley.
*e.* 2-inch iron grooved pulley.
*f.* slit crank.
*g.* set-screw attachment.

*Wooden-Grooved Pulleys.*—Their diameter is 6 inches, and width 2 inches. These are attached to the main shaft at intervals of 6 feet, and possess a groove about 9/16 inch in depth for a round belt, which drives the set of pulleys underneath the table. The length of the belt will depend on the proximity of the table to the main shaft. Patent snap-hooks for joining the belt are most convenient.

*Iron-Grooved Pulleys.*—Three are suspended by means of a small iron shaft (½x8 inches) in a pair of adjustable hangers (5½ inch) screwed underneath the table top. (1) The 8 inch (diameter) wheel gives 20 revolutions, that is, a respiratory rate of 20 per minute; (2) The 4 inch wheel gives a respiratory rate of 40 per minute; (3) The 2 inch wheel gives a respiratory rate of 82 per minute, and is practically suitable for insufflation. The small shaft extends out sufficiently for the attachment of a brass slit-crank, to which is attached a piston shaft. Fig. 2 shows a front view of the pulleys with hangers and slit-crank.

*Slit-Crank.*—The crank is 6½ inches long, ¾ inch wide and ⅜ inch thick, with a slit in the middle, extending the full length of the crank, except for the closed ends. Shifting the piston shaft up and down the slit provides for almost any desired change in volume of respiration, ranging from 240 c.c. to 1,250 c.c. in our standard pump. A set-screw attachment on the piston shaft permits an adjustment of the respiratory volume at a moment's notice by simply relaxing the belt, which is easily and readily secured by merely pushing the table in the direction of the main shaft. By means of this slit crank, and a suitably regulated T-tube in the tube leading to the trachea, we have successfully respired animals ranging from a small (250 gm.) guinea-pig to a 20 Kg. dog with the same pump.

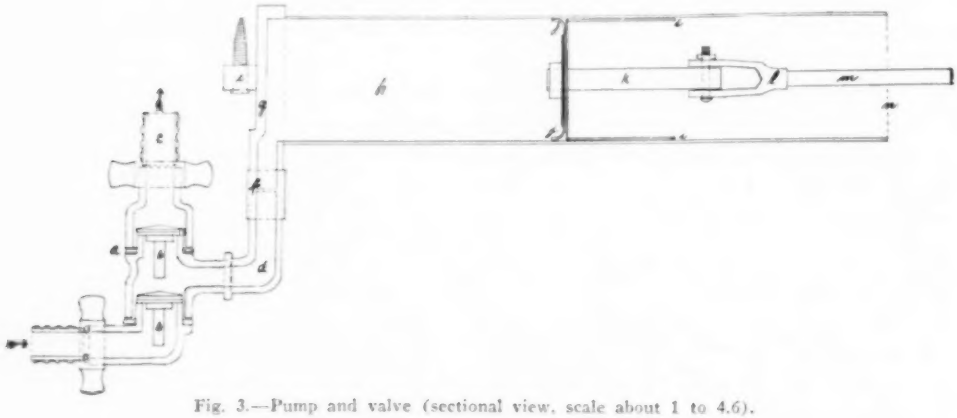


Fig. 3.—Pump and valve (sectional view, scale about 1 to 4.6).

*a.* valve.
*b.* brass pin.
*c.* air outlet, and rubber tubing attachment.
*d.* iron elbow.
*e.* projection with screw for fastening pump to front end of table.
*f.* brass union.
*g.* patent brass disc (distal end of cylinder).
*h.* cylinder.
*i.* piston-cup.
*j.* leather washer.
*k.* short piston tube.
*l.* fork attachment to piston shaft.
*m.* piston shaft.
*n.* open proximal end of cylinder.

*Piston-Shaft.*—This consists of a brass tube 40 inches long and ⅜ inch in diameter, with a set screw attachment to the slit-crank, and a fork attachment to the piston proper within the cylinder of the pump (Fig. 3).

*Cylinder.*—This consists of a brass tube 15 inches long and 3 inches in diameter, closed at the distal end with a patent brass disc, provided with a projection in the center for screwing to the table, and an outlet which communicates with the inside of the cylinder for the escape of air. The proximal end of the cylinder is left open to permit freer operation of the piston-shaft. The capacity of such a cylinder is 1,750 c.c. and this is our standard size. It is easily operated and answers for all practical purposes to which it may be put in artificial respiration. However, the entire capacity is not utilized, because a relatively

long piston-cup, which permits easy working of the piston attachments with the minimum of friction, is used.

*Piston.*—This is illustrated in Fig. 3, and consists of a brass cup 3 inches deep and 2⅞ inches in diameter, with a short stout brass tube 4x½ inches attached to the center of the bottom, to which is fitted a leather washer.

*Valve.*—The structure of this is sufficiently illustrated in Fig. 3, and simply consists of a chamber enclosing two short brass pins with their leather bases pointing upward, so that air is permitted to escape in one direction only. The valve is directly attached to the distal end of the cylinder by means of ordinary iron elbows used in joining gas or water pipes. This valve functionates in the upright or slightly oblique position only. Suitable ends on the valves are provided for the attachment of rubber tubing for the conduction of air through an anesthetizing attachment.

*Anesthetizing Attachment* (Fig. 1).—This consists of an ordinary Woulff bottle with two necks. By means of Y-pieces attached to the necks, ether or air can be passed at will by the regulation of screw stop-cocks placed on the rubber connections. The proximal Y-piece connects by means of a rubber tube (½ inch in diameter) with the valve. One limb of the Y-piece connects with a neck of the Woulff bottle, the other limb connects with a corresponding limb of the distal Y-piece, the other limb of which in turn connects with the other neck of the bottle. The distal Y-piece is now joined by means of rubber tubing to a T-piece, the distal end of which can serve as a tracheal cannula.

The Woulff bottle is easily secured in place by inserting it into a drilled wooden block permanently fastened to the corner of the table and situated close to the pump. The anesthetizing attachment permits of a considerable amount of motion, and all of its parts are easily detachable.

All of the attachments, with the exception of the piston cup and slit-crank, which have been described, are obtainable in the market. The valves and cylinders are obtainable from the Cleveland Faucet Company. The adjustable hanger and iron grooved pulleys can be secured from T. F. Welch & Co., Boston. The piston cup can easily be made by a mechanic of ordinary skill, and a slit-crank is most conveniently secured by having it specially cast.*

The advantages claimed for this apparatus may be summarized as follows: It is easily constructed, because all parts are easily obtainable in the market; it operates practically automatically; is easily adjustable; is serviceable for several different-sized animals at the same time; is relatively inexpensive and durable.

My thanks are due to our technician, Mr. Morris Dan, for aid in the construction of the apparatus, and to Mr. C. J. West, of the Second Year Class, for the drawings.

*The cost prices of the various parts and attachments for a unit are approximately as follows: Adjustable hanger, 90c; iron-grooved pulleys, 8-inch, 90c, 4-inch, 75c, 2-inch, 50c; slit-crank, 7c; piston-shaft, 15c; fork for piston-shaft, 5c; pump, including cylinder, piston and valve, $10.00; belting (5 ft.), 35c; accessories, about $1.25—total, about, $15.00. For the following articles used to operate five units, the cost was as follows: Used ¼ Hp. motor, $10.00; 30-ft. shaft, $3.00; 7 hangers and couplings for shaft at $1.50 per pair, $10.50; 5 wooden-grooved pulleys, at $1.00=$5.00; accessories, about $5.00. The total cost to set 5 units into operation was about $117.00.

# THE COLOR REACTIONS OF PROTEINS AND THEIR SPLIT PRODUCTS*

By Herbert W. Emerson, M.D., and John S. Chambers, M.S.,
Ann Arbor, Mich.

THE following experiments on the color reactions of some proteins and their split products were performed, with the hope that they might throw more light on the way in which the protein molecule is cleaved by Vaughan's method.[1] The variation in the intensity of the color reaction is expressed by varying the number of + signs in tabulating the results, and also, when possible, by determining the highest dilution that will give a positive color reaction.

TABLE I.

The Biuret test carried out in the usual way gives the following results:

| Dilutions. | 1-1000. | 1-5000. |
|---|---|---|
| Casein | + + + | + |
| Casein Poison | + + | + |
| Casein Residue | ± (1-100 +) | — |
| T. B. C. Germ Substance | + + + | ± |
| T. B. C. Poison | + + + | + |
| T. B. C. Residue | + | — |
| Colon Germ Substance | + + + | ± |
| Colon Poison | + + + | + |
| Colon Residue | ± (1-100 +) | — |
| Pepton | + + | — |
| Egg White | + + + | + |
| Dog Liver Poison | + + + | ± |

TABLE II.

Gies'[2] modification of the Biuret test gives practically the same results as the Biuret.

| Dilutions. | 1-1000. | 1-5000. |
|---|---|---|
| Casein | + + + | + |
| Casein Poison | + + + | — |
| Casein Residue | — (1-100+) | — |
| T. B. C. Germ Substance | + + + | ± |
| T. B. C. Poison | + + + | + |
| T. B. C. Residue | + | — |
| Colon Germ Substance | + + + | ± |
| Colon Poison | + + + | + |
| Colon Residue | + | — |
| Pepton | + + | — |
| Egg White | + + + | + |
| Dog Liver Poison | + + + | — |

TABLE III.

The Xanthoproteic test carried out in the usual manner gives results as follows:

| Dilutions. | 1-1000. | 1-5000. |
|---|---|---|
| Casein | + + + + + + | + + |
| Casein Poison | + + + + | + |
| Casein Residue | + | — |
| T. B. C. Germ Substance | + + + | — |

*From the Hygienic Laboratory of the University of Michigan.

TABLE III (CONT'D).

| Dilutions. | 1-1000. | 1-5000. |
|---|---|---|
| T. B. C. Poison | + + + + | ± |
| T. B. C. Residue | + + + | — |
| Colon Germ Substance | + + + | — |
| Colon Poison | + + + + | ± |
| Colon Residue | + + + | — |
| Pepton | + | — |
| Egg White | + + + + | + + |
| Dog Liver Poison | + + + | + |

TABLE IV.

Millon's test gave results as follows:

| Dilutions. | 1-1000. | 1-5000. |
|---|---|---|
| Casein | + + | ± |
| Casein Poison | + + + | + |
| Casein Residue | (Powder —) | — |
| T. B. C. Germ Substance | + + | — |
| T. B. C. Poison | + + + | + |
| T. B. C. Residue | (Powder —) | — |
| Colon Germ Substance | + + + + | + |
| Colon Poison | + + + + + | + + + |
| Colon Residue | (Powder —) | — |
| Pepton | + | — |
| Egg White | + + | — |
| Dog Liver Poison | + + + | + |

TABLE V.

Bardach's[3, 4] iodoform crystal test, in which the presence of proteins interferes with the formation of typical iodoform crystals, was carried out with very concentrated solutions of the proteins and the split products with the following results:

| | |
|---|---|
| Casein | + |
| Casein Poison | + |
| Casein Residue | — |
| T. B. C. Germ Substance | + |
| T. B. C. Poison | + |
| T. B. C. Residue | — |
| Colon Germ Substance | + |
| Colon Poison | + |
| Colon Residue | — |
| Pepton | + |
| Egg White | + |
| Dog Liver Poison | + |

TABLE VI.

The Molisch test, using only very concentrated solutions of the proteins and the split products, gives results as follows:

| | |
|---|---|
| Casein | + ? |
| Casein Poison | — |
| Casein Residue | + + + |
| T. B. C. Germ Substance | + + |
| T. B. C. Poison | — |
| T. B. C. Residue | + + + |
| Colon Germ Substance | + + |
| Colon Poison | — |
| Colon Residue | + + + + |
| Pepton | + |
| Egg White | + |
| Dog Liver Poison | — |

TABLE VII.

The Adamkiewicz reaction, using glacial acetic acid and concentrated sulphuric acid in the proportion 2:1 to which the dry powdered substance to be tested is added, gave the following results:

| | |
|---|---|
| Tyrosin* | + a pinkish violet color, |
| Tryptophane* | + + + a dark yellowish red, |
| Casein | + + light pinkish straw color, |
| Casein Poison | + + dark brownish red, |
| Casein Residue | + straw color with pink tinge, |
| T. B. C. Germ Substance | + + dark deep red, |
| T. B. C. Poison | + + brownish red, |
| T. B. C. Residue | + + + very deep red, |
| Colon Germ Substance | + + + + very deep red, |
| Colon Poison | + + + dark brownish red, |
| Colon Residue | + + + dark brownish red, |
| Pepton | + + light straw pink, |
| Egg White | + + + deep violet red, |
| Dog Liver Poison | + + dark brownish red. |

TABLE VIII.

The Hopkins Cole[5] reaction was carried out by adding a small amount of the powdered substance to be tested to three or four cubic centimeters of the Hopkins Cole reagent. Sulphuric acid was then run down the side of the test tube so as to form two layers.

| | |
|---|---|
| Casein | + + + pink ring, |
| Casein Poison | ? yellowish brown cloudy ring, |
| Casein Residue | + slight pink ring, |
| T. B. C. Germ Substance | + + pinkish violet ring, |
| T. B. C. Poison | ? yellowish brown cloud, |
| T. B. C. Residue | + pink violet ring, |
| Colon Germ Substance | + + + violet ring, |
| Colon Poison | ? yellowish brown cloud, |
| Colon Residue | + light pink ring, |
| Pepton | + + + reddish violet ring, |
| Egg White | + + + violet ring, |
| Dog Liver Poison | ? yellowish brown cloudy ring, no red or violet. |

TABLE IX.

The tests in Table VIII were repeated, adding one drop of furfural to each tube and thoroughly mixing its contents just before the sulphuric acid was run in.

| | |
|---|---|
| Casein | + + + + dark yellowish red ring, |
| Casein Poison | + + + + " " " |
| Casein Residue | + very slight yellowish pink ring, |
| T. B. C. Germ Substance | + + light yellowish red ring, |
| T. B. C. Poison | + + + " " " |
| T. B. C. Residue | + + + + heavy dark yellowish red ring, |
| Colon Germ Substance | + + + + + " " " |
| Colon Poison | + + + " " " |
| Colon Residue | + + + " " " |
| Pepton | + faint pinkish ring, |
| Egg White | + + + + yellowish violet ring, |
| Dog Liver Poison | + + + + " red " |
| Tryptophane | + + + + + " " " |
| Tyrosin | + very slight pinkish straw colored ring. |

TABLE X.

Benedicts' modification of the Hopkins Cole reaction gave the same results as is shown by the following:

| | |
|---|---|
| Casein | + + + reddish pink ring, |
| Casein Poison | ? brown cloudy ring, no red, |

*Controls were run by adding one drop of a one per cent solution of tyrosine or tryptophane and one drop of furfural to the acid mixture.

TABLE X (CONT'D).

| | |
|---|---|
| Casein Residue | + pink ring, |
| T. B. C. Germ Substance | + + violet ring, |
| T. B. C. Poison | ? brown cloudy ring, no red, |
| T. B. C. Residue | + + pink ring, |
| Colon Germ Substance | + + pink ring, |
| Colon Poison | ? light brown cloudy ring, no red, |
| Colon Residue | + pink ring, |
| Pepton | + + + bluish violet ring, |
| Egg White | + + + reddish violet ring, |
| Dog Liver Poison | ? brown cloudy ring, no red. |

TABLE XI.

The tests in Table X were repeated and modified by adding one drop of furfural to each tube and thoroughly mixing just before the sulphuric acid was run in. The following results were obtained:

| | |
|---|---|
| Casein | + + + dark yellowish red ring, |
| Casein Poison | + + + " " " " |
| Casein Residue | + very slight yellowish pink ring, |
| T. B. C. Germ Substance | + + light yellowish red ring, |
| T. B. C. Poison | + + " " " " |
| T. B. C. Residue | + + + heavy dark red ring with yellowish tinge, |
| Colon Germ Substance | + + + + yellowish red ring, |
| Colon Poison | + + + " " " |
| Colon Residue | + + + " " " |
| Pepton | + + faint pinkish ring, |
| Egg White | + + + + yellowish violet ring, |
| Dog Liver Poison | + + + + yellowish red ring. |
| Tyrosine | + very slight pinkish straw colored ring, |
| Trytophane | + + + + + yellowish red ring. |

TABLE XII.

The Acree Rosenheim formaldehyde test was carried out by adding two or three drops of a 1-5000 formaldehyde solution to about four cubic centimeters of strong aqueous solution of the substances to be tested and thoroughly mixing before running in the sulphuric acid.

| | |
|---|---|
| Casein | + + violet ring, |
| Casein Poison | ? brown cloudy ring, no red, |
| Casein Residue | + pink ring, |
| T. B. C. Germ Substance | + faint violet ring, |
| T. B. C. Poison | ? brown cloudy ring, no red, |
| T. B. C. Residue | + + light pink ring, |
| Colon Germ Substance | + + pink ring, |
| Colon Poison | ? brown cloudy ring, no red, |
| Colon Residue | + + pink ring, |
| Pepton | + + + bluish violet ring, |
| Egg White | + + reddish violet ring, |
| Dog Liver Poison | ? brown cloudy ring, no red. |

TABLE XIII.

The tests in Table XII were repeated and modified by adding one drop of furfural to each tube and thoroughly mixing before the sulphuric acid was run in. The results obtained were as follows:

| | |
|---|---|
| Casein | + + + yellowish red ring, |
| Casein Poison | + + + " " " |
| Casein Residue | + + pink ring, |
| T. B. C. Germ Substance | + + yellowish red ring, |
| T. B. C. Poison | + + " " " |
| T. B. C. Residue | + + + " " " |
| Colon Germ Substance | + + " " " |

TABLE XIII (CONT'D).

| | |
|---|---|
| Colon Poison | + + yellowish red ring, |
| Colon Residue | + + " " " |
| Pepton | + faint pink ring, |
| Egg White | + + brownish red ring, |
| Dog Liver Poison | + + yellowish red ring. |

TABLE XIV.

The benzaldehyde test, in which the development of a green to a blue color indicates the presence of tryptophane, was carried out as follows: equal volumes of concentrated hydrochloric acid and a two or three per cent solution of the substances to be tested were mixed in a test tube, one drop of ferric chloride and two drops benzaldehyde were added and the contents heated to boiling.

| | |
|---|---|
| Casein | + + + + intense blue color, |
| Casein Poison | — black oily residue, no blue or green color, |
| Casein Residue | + light yellowish green, |
| T. B. C. Germ Substance | + + + + sky blue. |
| T. B. C. Poison | — black oily residue, no blue or green color, |
| T. B. C. Residue | + dark bluish green, |
| Colon Germ Substance | + + + light blue with yellow tinge, |
| Colon Poison | — brown liquid, oily residue, no blue or green, |
| Colon Residue | + + dark brownish green, |
| Pepton | + brown precipitate with green tinge, |
| Egg White | + + + intense sky blue, |
| Dog Liver Poison | — black oily residue, no green or blue. |

SUMMARY.

1. The proteins and their split products, the protein poisons and the residues, all give the biuret reaction. The residues will not give the reaction in dilutions much greater than (1-100). This indicates that the proteins and their split products contain an acid amide group and other substituted amide groups attached to neighboring carbon atoms, and that the end products have not been deamidized in cleaving.

2. Gies' biuret reagent gives similar results.

3. The proteins and their split products all give the xanthoproteic reaction and the poisons in greater dilutions than the proteins or the residues, indicating that they all contain benzene nuclei and that in cleaving these tend to concentrate in the poison.

4. The residues do not give Millon's reaction and the poisons give it in greater dilutions than the proteins indicating that all of the mono-hydroxy-benzene nuclei (tyrosine) are cleaved off into the poison. It is interesting to note the strong reaction given by the colon cell substance and by the poison.

5. The residues do not give Bardach's reaction and all soluble proteins are said to give it. The residues are alkaline and soluble.

6. The poisons do not give the Molisch reaction and the residues give a stronger Molisch than the proteins indicating that the carbohydrate groups are cleaved off into the residues. Casein gives a very weak Molisch reaction and casein residue a strong reaction, this may be due to tryptophane, but we think not, as the casein residue gives only a weak tryptophane reaction. Casein residue does not reduce Fehling's solution even after boiling with dilute hydrochloric acid. Part of the residue is insoluble in 10 per cent alkali and this insoluble part gives the Molisch reaction and also tests for nitrogen.

TABLE XV

| COLOR REACTION | CASEIN | CASEIN POISON | CASEIN RESIDUE | T. B. C. GERM SUBSTANCE | T. B. C. POISON | T. B. C. RESIDUE | COLON GERM SUBSTANCE | COLON POISON | COLON RESIDUE | PEPTONE | EGG WHITE | DOG LIVER POISON |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biuret | (1-5000) + | (1-5000) + | (1-100) + | (1-5000) ± | (1-5000) + | (1-1000) + | (1-5000) ± | (1-5000) + | (1-100) + | (1-1000) ++ | (1-5000) + | (1-5000) ± |
| Gies' Biuret | (1-5000) ++ | (1-1000) +++ | (1-100) + | (1-5000) ± | (1-5000) + | (1-1000) + | (1-5000) ± | (1-5000) + | (1-1000) + | (1-1000) ++ | (1-5000) + | (1-1000) +++ |
| Xanthoproteic | (1-5000) ++ | (1-5000) + | (1-1000) + | (1-1000) +++ | (1-5000) ± | (1-1000) +++ | (1-1000) +++ | (1-5000) ± | (1-1000) +++ | (1-5000) + | (1-5000) ++ | (1-5000) + |
| Millon's | (1-5000) ± | (1-5000) + | Powder — | (1-1000) ++ | (1-5000) + | Powder — | (1-5000) + | (1-5000) +++ | Powder — | (1-1000) + | (1-1000) ++ | (1-5000) + |
| Bardach | + | + | — | + | + | — | + | + | — | + | + | + |
| Molisch | — | — | + | ++ | — | +++ | ++ | — | ++++ | + | + | — |
| Adamkiewicz | ++ | ++ | + | ++ | ++ | +++ | ++++ | +++ | +++ | ++ | +++ | ++ |
| Hopkins Cole | +++ | ? | + | ++ | ? | + | +++ | ? | + | +++ | +++ | ? |
| Hopkins Cole & Furfural | ++++ | ++++ | + | ++ | +++ | ++++ | +++++ | +++ | +++ | + | ++++ | + |
| Benedict's Hopkins Cole | +++ | ? | + | ++ | ? | ++ | ++ | ? | + | +++ | +++ | ? |
| Benedict's Hopkins Cole & Furfural | +++ | +++ | + | ++ | ++ | +++ | ++++ | +++ | +++ | ++ | ++++ | ++++ |
| Acree Rosenheim Formaldehyde Test | ++ | ? | + | + | ? | ++ | ++ | ? | ++ | +++ | ++ | ? |
| Acree Rosenheim Test + Furfural | +++ | +++ | ++ | ++ | ++ | +++ | ++ | ++ | ++ | + | ++ | ++ |
| Reichl's Benzaldehyde Test | ++++ | — | + | ++++ | — | + | +++ | — | ++ | + | +++ | — |

7. The proteins and their split products all give the Adamkiewicz reaction indicating that they all contain the tryptophane group. Solutions of tyrosine give pinkish violet colors which are similar to the tryptophane reactions in dilute solutions.

8. The protein poisons do not give good Hopkins Cole reactions when performed in the regular way. The proteins and residues do. They all give good positive reactions when furfural is added. The protein poisons probably give negative reactions because they contain no carbohydrate.

9. Benedict's modification of Hopkins Cole reagent gives similar results both with and without furfural.

10. The Acree Rosenheim formaldehyde test gives similar results to those obtained with the Hopkins Cole reagent both with and without furfural.

11. The proteins and the residues give positive results with the benzaldehyde test (Reichl's reaction). The poisons give negative reactions and the addition of furfural has no effect.

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# *LABORATORY METHODS*

## PHYSICAL CHARACTERISTICS OF SPINAL FLUID IN VARIOUS DISEASES*

By A. Levinson, M.D., Chicago, Ill.

WITH the advance of our knowledge of the chemistry of spinal fluid, we are gradually losing sight of the physical characteristics of the fluid. This is unfortunate, as the physical properties are often valuable in establishing the diagnosis and even the prognosis of the disease, sometimes being as reliable as a chemical test. I should like to point out a few of these characteristics that seem to be of clinical importance.

### APPEARANCE OF FLUID IMMEDIATELY AFTER WITHDRAWAL FROM THE BODY.

It is well known that normal fluid is clear and colorless and that meningitic fluid is turbid in appearance. It is not well known, however, that different types of meningitis impart a rather characteristic color to the fluid. Repeated observations have convinced me that while the color differs with the consistency of the fluid which in turn is in constant ratio to the stage of the disease, there is a characteristic tinge that is present in most cases in all stages of the disease unless altered by serum.

The fluid in epidemic meningitis is yellowish in color, with a greenish tinge in it. The more advanced the disease, the more yellow the color of the fluid. At times the fluid will turn green of its own accord. This has happened in a number of our cases (when no contamination has taken place).

When a sediment forms in the meningococcic variety the supernatant fluid still remains yellowish in color.

The fluid in pneumococcus meningitis is pearl gray in color. Even after the sediment has formed, the supernatant fluid remains grayish in color. When serum is administered the fluid takes on a yellowish tinge, but not as marked as in the epidemic variety.

In tuberculous meningitis the fluid is colorless, even when it is turbid in consistency it remains colorless.

### SEDIMENT.

It is a well known fact that the fluid of tuberculous meningitis forms a pellicle on standing. Lichtheim described this years ago. It is not so generally known, however, that a pellicle or rather a sediment makes its appearance in every form of meningitis. In fifty cases, that I observed with this in view, I noted the formation of a sediment in practically every case of meningitis. Furthermore, each form of meningitis shows a sediment peculiar to its type, which

*From the Sarah Morris Hospital for Diseases of Children and from The Nelson Morris Institute for Medical Research, Chicago, Ill.
Read before the Chicago Medical Society, March 8, 1916.

if properly observed may become helpful in establishing the direct as well as the differential diagnosis.

The sediment formation makes its appearance within not later than twenty-four hours. In meningococcic and pneumococcic meningitis, however, the pellicle or sediment forms quite rapidly, usually as early as one-half hour after the removal of the spinal fluid from the body or even earlier. The sediment is likely to appear under any conditions; however, the conditions most favorable for its formation and preservation, are the following:

1. The spinal fluid should be left undisturbed.
2. The tube containing the fluid should be tightly corked. The latter precaution is not as essential as the former.

In order to facilitate the formation of a pellicle, and in order to obtain uniform results with the other tests on the spinal fluid, it is necessary to have a uniform system in the taking of the fluid. For this purpose I should strongly recommend the adoption of the following plan which I have treated more fully elsewhere (*Medical Record*, lxxxix, No. 17). I shall here mention merely the essential features of the plan.

Four sterile and chemically clean test tubes of uniform size are taken, though in most cases three are sufficient for complete examination. The fourth, however, comes in handy if the quantity of fluid is very large. The tubes should be labeled, 1, 2, 3, 4, and should preferably be calibrated.

Tube 1 should receive the first 2 c.c. of fluid, and this amount should be used for.

1. Cytological examination.
2. Direct uncentrifuged smear for microorganisms.
3. One-half c.c. should be used for culture.
4. One c.c. should be used for the permanganate test (the index for organic substances in the spinal fluid). If not examined immediately this test tube and its contents should be placed in the incubator.

Into the second tube should be run from 3 to 5 c.c. of spinal fluid, depending upon the amount and the pressure of the fluid although 5 is preferable. This should be put into a tube rack, and placed in the refrigerator for the formation of a pellicle or sediment, care being taken that the tube is not disturbed. It is best to use a regular cork instead of a cotton plug. This will not only facilitate the formation of a pellicle, but will also preserve the fluid better than will the cotton plug.

Into the third tube should be run from 5 to 8 c.c. of fluid to be used for

1. The second portion of the permanganate test.
2. The different globulin tests (Noguchi, Ross Jones and Nonne).
3. Lange.
4. Wassermann.
5. Sugar contents.
6. Ten c.c. for centrifugation, for examination of the organism if the first portion did not show it.
7. All other tests that one may desire to perform. Into the fourth tube should be run the rest of the spinal fluid desired either for examination or for

the purpose of relieving pressure which is from 5 to 15 c.c. on the average if the case is one of meningitis.

TYPES OF SEDIMENT.

I. The spinal fluid of meningococcus-meningitis is turbid on withdrawal resembling the color of very rich cream. A sediment or pellicle forms in from one-half hour to one and one-half hours later depending upon the stage of the disease.

(a) In the early stages the pellicle is made up of a yellowish white network, the reticuli being close together forming an opaque layer. The upper portion of the network is balloon or dome-shaped, and the base or lower portion is flattened in appearance; the whole network resembling a small bursa. The network usually does not reach the upper level of the fluid, and as a rule, it inclines toward one side of the tube. The pellicle differs from the one formed in tuberculous meningitis in the following particulars:

1. The pellicle is not suspended in the center.
2. There are no processes projecting from the side of the pellicle.
3. The color is more yellowish and the network more firm.
4. The fluid remains serum-like in color and consistency after the formation of the sediment.

(b) In the more advanced stage of meningitis as when the disease has progressed for a number of days, the turbidity upon withdrawal may or may not be marked, but the sediment formation is firmer, resembling a membrane in its appearance. It usually spreads out along one side of the tube, in the form of a heavy film (Fig. B).

(c) In still more advanced cases, there is a sediment that sinks to the bottom, but still as a rule, no coagulation occurs. In this stage a deposit of yellow granules is seen, attached to one side of the tube. These granules may be considered as a manifestation of an advanced stage (Fig. C).

(d) In the very grave cases the sediment is thick and falls by gravity to the bottom. In the cases where this happened and when the pellicle was more than three-fourths inch in height, the prognosis as a rule has been grave (Fig. D).

When the meningitis recedes, the pellicle or sediment takes on a character the reverse of the one that appeared at its formation, often changing from membranous deposit back to a reticular formation (Fig. E).

II. In pneumococcic meningitis, the fluid is usually not so turbid as in meningococcic, and it is grayish in color, a pellicle or sediment forming in one-half hour or more.

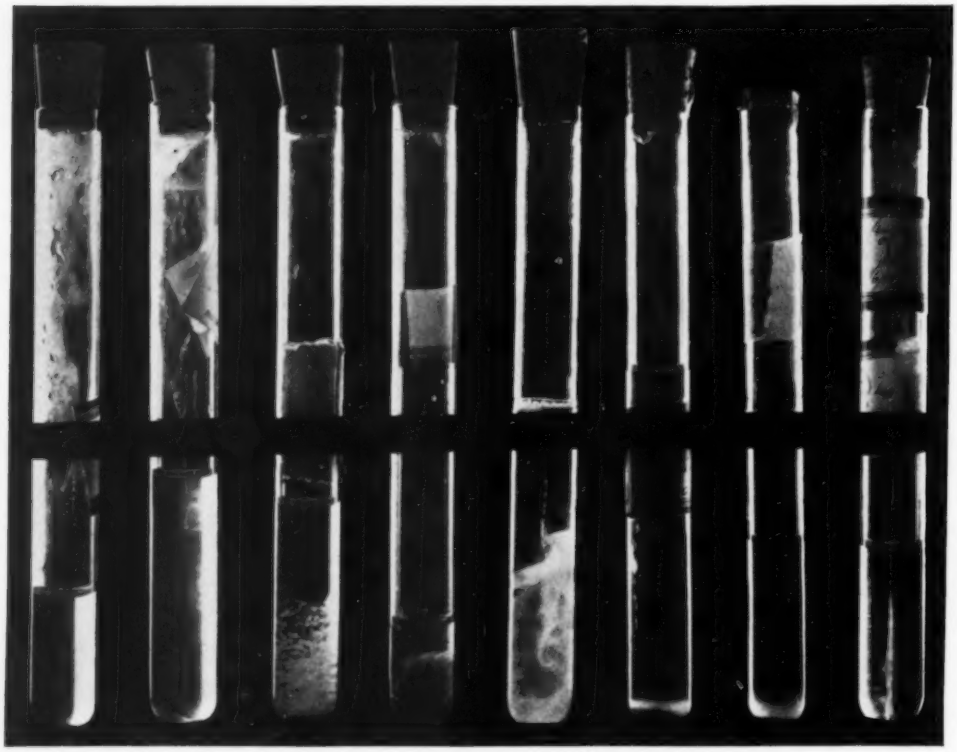
(a) In the early stage a pellicle forms which is triangular in shape and hangs suspended in the center of the tube. The pellicle which is grayish in color is firm and made up of fibrous masses. It differs from the T. B. pellicle in that it is more opaque, firmer, and more grayish in color. It differs from meningococcic sediment in that it is not as flattened, and is more firm than the latter.

(b) In the advanced stages the sediment sinks to the bottom in a homogeneous mass. Within five or six hours, as a rule, the sediment coagulates and

takes on the appearance of the boiled white of an egg (Figs. F and G), around the center of a noncoagulated mass.

III. T. B. meningitis forms a pellicle in from six to twelve hours either in the form of a white streak with small projections from the sides, or in advanced cases, in the shape of a fine, triangular-formed network, with thin reticuli, the apex of the sediment touching the surface of the fluid, and the base touching the bottom (Fig. H). The structure is not firm and rough handling will break it or cause it to curl up at the bottom of the tube. The pellicle is always snow-white in color, unless there is a mixture of blood in the tube, and is made up of fine reticuli. The rest of the fluid is clear, even if before the appearance of the sediment it appeared to be slightly turbid.

Often when a T. B. meningitis is mistaken for other forms of meningitis



*A* *B* *C* *D* *E* *F* *G* *H*

PELLICLE FORMATION IN MENINGITIS.

*A.* Normal.
*B.* Meningococcic meningitis, fairly early.
*C.* Meningococcic meningitis, advanced (sulphur-like granules).
*D.* Meningococcic meningitis, late in disease.
*E.* Meningococcic meningitis, severe, on road to recovery.
*F.* Pneumococcic meningitis, fatal.
*G.* Pneumococcic meningitis, fatal.
*H.* Tuberculous meningitis.

and serum is administered the pellicle will still form if the fluid has been left undisturbed. The pellicle formation is of diagnostic value in such cases, since the serum injection renders all chemical tests valueless.

The pellicle formation is of great assistance diagnostically when the spinal

fluid withdrawn is mixed with blood, and all other tests but the bacteriological are thereby rendered useless. The sediment appears in a short time directly above the blood that has settled at the bottom, showing the same characteristics as if no blood were present.

I made an attempt to study the microscopic appearance of the sediment in the various forms of meningitis. The superficial examination of the sediment in meningococcic meningitis showed leucocytes and reticuli. In one case of pneumococcic meningitis the microscopical examination showed fibrinous masses, in another homogeneous masses. I can at present say nothing positive, however, about the microscopic character of the sediment. I hope to be able to go into the investigation more thoroughly in the near future.

FOAM.

I observed a rather peculiar thing in the specimens of spinal fluid that came under my observation—a phenomenon to which I have not yet been able to find any reference in literature—namely the formation of a foam on the surface of pathological specimens of spinal fluid. With one exception I found that all the pathological fluids, covering every form of meningitis and of lues, produced a foam when the tube was shaken even slightly, whereas in the case of normal fluids even the most vigorous shaking did not result in the formation of a thick foam, the utmost being the rise of a thin layer of bubbles to the surface of the fluid. The foam produced in the pathological fluids I found to be from ½ to 2 inches in height, resembling a soap foam in all its details. The foam thus formed persists from one-half to several hours, when left undisturbed, while the bubbles produced in the normal spinal fluid disappear in a very short while. What adds greater significance to the foam formation is the fact that the alkalinity of the spinal fluid that foamed was increased, upon standing, from one-half to one and one-half c.c. of a n/100 $H_2SO_4$, above the alkalinity obtained right after the withdrawal of the fluid from the body. (The indicator I used was methyl red which I described more fully in the *Archives of Pediatrics,* April, 1916.) The fluid that did not foam showed no increase in alkalinity upon standing. Whether this will hold good in every case is something that remains for investigators in this field to determine. If it does, it may serve as a rough index for pathological fluids.

SUMMARY.

1. The color of the fluid varies with the form of meningitis.
2. A sediment appears in every form of meningitis.
3. The sediment forms most rapidly when the fluid is left undisturbed.
4. The sediment appears in ½ to 12 hours after withdrawal from the body, forming most quickly in meningococcic and pneumococcic meningitis.
5. In meningococcic meningitis the sediment appears at first in the shape of a balloon-like mist, and later takes on a film-like form.

It appears attached to one side of the tube, as a rule, not reaching the surface of the fluid. As the disease advances the sediment drops to the bottom.

6. Pneumococcic meningitis gives a grayish white pellicle triangular in form in the early stages of the disease and a sediment that sinks to the bottom in

the advanced stage. In the latter case the sediment coagulates in a short time. The fluid remains grayish in color.

7. Tuberculous meningitis gives a pellicle formation which extends from the upper level to the bottom of the test tube. It appears in either a thread-like or cone-like formation, with small processes projecting sideward. The pellicle is snow-white in color (unless there is blood in it).

8. A pellicle forms in spite of serum injection.

9. A pellicle forms when the spinal fluid is mixed with blood.

10. A foam appears at the surface of fluids drawn from pathologic cases when the tube is but slightly shaken, and persists for some time.

11. A uniform method of taking spinal fluid facilitates all tests on spinal fluid.

## THE "DELAYED NEGATIVE" WASSERMANN REACTION

By George Manghill Olson, M.D., Minneapolis, Minn.

THE Wassermann reaction is not infrequently negative when the patient shows signs of syphilis or very obviously has not had sufficient treatment. In late or tertiary syphilis the reaction may be negative when the patient has had no treatment at all. In order to increase the percentages of positives in syphilis, and to render the test more delicate, the Wassermann reaction has been modified by a large number of methods, and attempts at its replacement by other and entirely different tests have been made. The unfortunate result of this attempt to modify or cause the Wassermann test to give more positives in syphilis has been not only to cause more positives in syphilis, but to get positives in patients that were entirely free from syphilis. So that today the original Wassermann test is still the most useful, and is less liable to error than the many methods designed to replace it.

The "delayed negative" Wassermann does not modify the technic in any way, and is made simply by taking readings at twenty minute intervals while the tubes are in the incubator, instead of one reading at the end of two hours. The Wassermann test showing complete hemolysis at the end of two hours is read as negative. This hemolysis however often occurs in twenty to thirty minutes after the tubes have been placed in the incubator. The time at which this hemolysis occurs varies very markedly when testing different sera, and if only one reading is taken at the end of two hours, much valuable information will have been lost. Readings should be taken at intervals of twenty minutes, and if this is done, the "delayed negative" Wassermann will often be found. At the end of the first twenty minutes, often the negative control will be completely hemolyzed, there will be no hemolysis in the positive control, and the serum to be tested will show no hemolysis. In other words the serum to be tested shows a positive reaction at the end of the first twenty minutes. At the end of forty minutes, however, the serum to be tested shows marked hemolysis, while the positive control still shows absolutely no hemolysis. At the end of two hours the serum to be tested is completely hemolyzed or absolutely negative.

I have found this "delayed negative" Wassermann to be of very much greater value than the slightly positive Wassermann, where the test shows only some non-hemolysis at the end of two hours in the incubator. The slight positive Wassermann is often due to spoiled serum, errors in technic, etc. The "delayed negative" Wassermann is, I believe, of much greater value in showing the presence of some of the so-called antibodies of syphilis, even when they are not present in sufficient amount to give a positive Wassermann. In patients with the initial lesion of syphilis, this reaction is often present when the Wassermann reaction is absent. Any patient having a suspicious sore, and showing this "delayed negative" Wassermann, should be very carefully and repeatedly examined for the presence of the Spirocheta pallida. The reaction is also of importance in the tertiary stage, where patients with active lesions of syphilis show thirty-three per cent of negative Wassermann reactions. As a guide in the treatment of syphilis, the "delayed negative" is an indication for more treatment, even though the Wassermann is completely negative at the end of two hours in the incubator.

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## EDITORIALS

### *The Etiology of the Common Cold*

OF all the less serious diseases that afflict human beings, there is unquestionably none that leads to so much discomfort and that, economically speaking, reduces efficiency by a higher percentage than the so-called "cold in the head." There seems to be no lasting immunity and it goes through families, schools and institutions in a way that has long left no doubt as to its transmissibility from person to person. It has been investigated bacteriologically by a large number of workers, probably by many more than the literature shows. We alone know of two pieces of work done on this illness, which were never published because of their inconclusiveness. When the colds have been very severe, they have usually been called "grippe" or "influenza," in spite of the fact that the Pfeiffer bacillus was not often found. At various times, the influenza bacillus, the different streptococci and pneumococci have been held responsible. During recent years Tunnicliff described a bacillus to which she attributes etiological importance. It has been noticed by many bacteriologists, who have studied the condition, that the mucus from typical colds was curiously free from bacteria.

In 1914 Kruse[1] suggested that the ordinary cold might be due to a filterable

[1]Kruse: Munchener Mediz. Woch., 1914, vol. lxi.

virus. He filtered the secretions from the nose of a person suffering from a cold, through a filter impervious to bacteria, proved it free of bacteria and, with it, inoculated a series of volunteers, one-third of whom contracted colds within one to four days. In a second series of 36, he obtained positive results in 42 per cent.

Foster of the U. S. Army Medical Corps has recently repeated Kruse's experiment in the Laboratory of Preventive Medicine at Harvard. The case with which he began work was one of typical coryza with moderate systemic symptoms and profuse mucoid nasal discharge. From this and two similar cases, secretion was obtained, mixed with isotonic salt solution, and filtered through a Berkfeldt "N" filter. Bacterial cultures of this filtrate proved sterile. Ten volunteers from the garrison of Fort Banks were inoculated with the filtrate by instilling about three to six drops into each nostril, after washing the nose with water. Nine of these ten developed symptoms of acute coryza in from eight to thirty hours. Some of them developed simple rhinitis, others had slight fever, with involvement of the eustachian tubes, sore throat, cough, and pain in the back; one of them had tenderness of the parotid, another herpes of the lips. These cases of Foster seem definitely to confirm Kruse's observation and, in the light of these results, we may be permitted to add a single instance observed by Dr. Hopkins and the writer, which appears now to have more importance than we ventured to attribute to it at the time. A secretion, filtered and controlled just as were those of Foster, was obtained from the nasal mucous of a student at Columbia, suffering from acute coryza, and about 1 c.c. instilled into the nose of a member of the laboratory staff. He developed a violent coryza within three days. However, this individual had been exposed, as had the rest of the laboratory staff, to intimate contact with the class, a high percentage of whom were suffering from acute coryza at the time, and control experimentation could not, therefore, be carried out. This is mentioned only because of the fact that it seems to us of great interest in connection with Foster's excellent investigation.

Particular importance is lent to Foster's work by the fact that he went on to the cultivation of the apparently filterable virus by the methods employed by Flexner and Noguchi in the case of poliomyelitis, in which tissue ascitic fluid and tissue broth were used in high tubes sealed with paraffin oil. About 0.5 c.c. of the nasal filtrate was introduced into the tubes, directly in contact with the tissue, after these tubes had been proved sterile by previous incubation. A distinct, grayish-white, opalescent halo appeared at the end of twenty-four hours, in the ascitic fluid and broth. This thickened for three or four days and the haziness extended up the tubes. Under the dark field, active, minute bodies, occurring singly and in groups, appeared and, stained by various methods, minute coccoid bodies were demonstrable in all the preparations. Successful inoculations were obtained from eleven soldiers into whose noses the filtrates obtained from sub-cultures and so diluted that the original material obtained from the first case was in concentration not higher than 1,000 to 90,000.

We regard the work of Foster as of unusual importance. His results are clear-cut and, although in the original conception a confirmation of Kruse, go much further in that they may possibly have resulted in the successful cultivation of the microorganism. It is further worthy of comment that Foster's work,

though systematically and very accurately carried out, is presented with a degree of conservatism and self-criticism which lends additional weight to the conclusions which he himself regards as justified.

—*H. Z.*

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## *Death by Suffocation*

DEATH from suffocation may result in the following ways:

1. From the growth of a tumor in the larynx or trachea.

2. By disease of the glottis, such as edema, or by spasm produced by some powerful irritant, such as boiling water or some corrosive poison. These modes of death by suffocation may be natural or due to disease.

3. The accidental entrance of foreign bodies into the larynx. There are many cases, especially among children in which death has been caused by bits of food getting lodged in the windpipe during swallowing, or by vomited matter getting into the windpipe.

Mackenzie tells of a man who choked while attempting to swallow a large piece of meat. Death occurred in a short time, and postmortem examination showed that the piece of beef had lodged in the glottis, a part of it having passed through the rim. In another instance a drunken man was found dead, and examination showed a thin piece of potato skin covering the rim of the glottis so closely that it prevented the entrance of air. The man had eaten potatoes for dinner, and while thoroughly intoxicated vomited, and being unconscious he was unable to cough, and death resulted from this mechanical obstruction. In still another instance cited by Taylor a woman died during a violent fit of coughing. An autopsy showed a large tough piece of mucus lodged in and closing the rim of the glottis.

4. A foreign body may be forcibly introduced into the mouth and crowded down upon the glottis so as to cut off the air supply. The introduction of such bodies may be done without the purpose of injury. Thus, nurses sometimes gag their charges with little bags made of soft leather or cloth containing some sweet substance. In several instances death has resulted in this way. The child, unable to remove the gag, carries it back into its throat until respiration is interfered with, and death may be induced. In some instances this gag is only a rag sweetened with sugar.

5. By too closely covering the head. It is easy to kill an infant by suffocation either accidentally or intentionally. The writer has known of several instances in which children only a few days old have gone to sleep closely pressed to the mother's breast. The mother also has fallen asleep, the child's head gets under the bedclothes, or is in part covered by the body of its mother, and it dies without a struggle. This is known as death from smothering or overlaying. In the great majority of instances it is unintentional, but cases of intentional infanticide by this method have been reported. Any person may be killed by smothering, provided the difference in strength between the victim and the murderer is sufficient to allow the latter to accomplish his purpose. In order to render his victim more helpless the homicide may resort to the previous

administration of some drug. As a rule homicidal death by smothering is inflicted only upon infants or the aged and infirm.

6. By accidentally burying the body in grain, flour, ashes, or some other finely divided substance. A man may fall down a wheat elevator, escape all violence from the fall, but may die in a few minutes from suffocation. Deaths caused in this way are probably always accidental.

7. The face may be held in snow, mud, ashes, feathers, grain, leaves, or some similar substances until death from suffocation results.

8. The hand or a cloth may be forcibly held over the mouth or nostrils until death results. Frequently the homicide in resorting to this method aids in the accomplishment of his purpose by placing the weight of his body upon the chest of his victim.

It is quite evident from what has been said that even homicidal death by suffocation may result without any external evidences of injury. In investigating deaths from suffocation, the expert must carefully note the condition of the larynx, and especially look for the presence of foreign bodies in this location. Tardieu once believed that the presence of minute ecchymoses on the surface of the lungs beneath the pleura was characteristic of death by suffocation. He described these spots as varying in size from that of a pinhead to a lentil, and in number from five or six to so many that the lung had a granite-like appearance. They are found most abundantly near the base of the lungs. However, other investigators claim that these ecchymoses although common in death from suffocation, are met with in other forms of asphyxia, and a committee of the medico-legal society of Paris made the following report concerning them:

1. Subpleural ecchymoses may appear after death from various causes:

2. They may be met with after death from hanging, strangulation, submersion, and suffocation:

3. They are of value only when associated with other signs indicating the cause of death.

The condition of the heart is variable, but as a rule corresponds with that found after death from other forms of asphyxiation. The blood is fluid and the right heart distended, while ecchymoses are frequently found in the pericardium. The brain as a rule is engorged with venous blood, and there may be hemorrhages, although these are rarely found.

Suffocation is the cause of death in most persons killed by crowding. When people are seized with panic and rush from a building most of those who lose their lives are suffocated and not trampled to death. The body is so compressed by the crowd and the volume of air so limited, that death from suffocation results. This may happen, if the crowd be sufficiently dense, in the open air. Indeed, the most striking illustrations of this kind of death have occurred in the open, as for instance the death of more than 1,500 persons in the open plain near Moscow at the time of the coronation of the present Emperor of Russia.

*Suffocation from Disease.*—It should always be borne in mind that every sudden death from suffocation is not due to violence, either suicidal or homicidal, nor to accident; and whenever an obduction is held the man or men who perform it should see whether or not death may have been due to some patho-

logical condition. The presence of a tumor is so easily recognized that it needs only to be mentioned. Laryngeal diphtheria, fortunately much less common now than before the discovery and application of diphtheria antitoxin, is still an occasional cause of sudden death, although as a rule death is preceded by some days of illness. It is known that a small plug of bronchitic mucus may suffice in infants to close one of the small bronchi and cause sudden death. In some instances this obstruction to the passage of air is so small that it can be detected only by careful microscopic examination. Herford, as quoted by Ziemke, reports six cases of sudden death in children in which no macroscopic cause could be found, but histological examination proved the existence of capillary bronchitis. It seems that there are instances in which the thyroid enlarges without attracting attention and finally interferes with respiration and may cause sudden death by pressure on the blood vessels or by reflex action through the nerves. Richter thinks that in these instances there may be marked hyperemia and swelling in the tracheal mucous membrane. Again, there is the condition known as status lymphaticus in which there is general hyperplasia of the lymphatics, involving the thymus, the solitary and agminated glands in the mucous membrane of the intestines and the lymphatic glands in other portions of the body. It has been supposed by some that the hyperplastic thymus in children through either a very rapid enlargement or by pressure being brought to bear on some nerve by a sudden movement may sometimes cause sudden death. These explanations are largely hypothetical and not altogether satisfactory. Ziemke states that in some of these cases he has found catarrhal changes in the lungs and more frequently in the intestines. In the instance of a child one year of age that had repeatedly shown signs of heart weakness and had suffered from intestinal catarrh, he found hyperplasia of the thymus with ecchymoses, a marked status lymphaticus, an extraordinary dilatation of the left ventricle and a fatty degeneration of the cardiac muscle. He also states that in cases of sudden death in children in which evident changes are lacking, he has never failed to observe a well developed rachitis. In such instances there may be the condition designated by Kassowitz as "expiratory apnea" and spasm of the muscles of the glottis may account for the sudden death. The theory of a suddenly developed endogenous poison is of special interest in connection with the recent discovery of the fact that many proteins contain a poisonous group, but much work along this line must be done before positive statement can be made. The subject of a sudden death in early life with the literature has been admirably presented by Griffith.[1]

Sury[2] gives an extensive review of the literature of so-called *mors thymica*, adds considerable of value from his own studies, and reaches conclusions that may be abbreviated as follows: None of the reports of cases of death from this cause in the newly born are free from error. Aspiration of fluid into the bronchi may happen in so-called easy births when the pains follow in rapid succession. The fetus suffers from oxygen hunger and breathes too soon. As a rule, the size of the thymus varies with that of the child, and in many instances in which the gland has been reported as "enormous" its weight falls within normal limits. Sudden death of a child from mechanical causes in case of normal thymus have not been demonstrated. There is in these cases a natural cause of death, and this is generally either a bronchitis or an enteritis, which

is overlooked at the obduction. Chronic difficulty of respiration, as continued stridor and partial asphyxiation, rarely, though sometimes, seen in children, is benefited by partial or total extirpation of the thymus. The explanation of this is not clear. In only one case, that of Rolleston, was the gland found to weigh more than 300 g. Complete closure of the trachea in cases of so-called enlarged thymus by throwing the head backwards, has not been proven. Dangerous pressure on the vessels or nerves by a thymus of normal size has not been demonstrated, and seems improbable. The so-called typical signs of asphyxiation are not pathognomonic, and consequently are not of value in forensic medicine. A positive diagnosis of status lymphaticus in a child is difficult, and should be made only after a most careful study. The involution of the thymus begins with adolescence, and there is a relation between this gland and the sexual organs not yet understood.

*Suffocation in Attempting to Swallow.*—When one attempts to swallow something that is too large to pass into the esophagus, it may get so wedged into the throat as to occlude the larynx. Unless the object is speedily dislodged, dyspnea, spasm and loss of consciousness followed by death may follow. The pieces that men have attempted to swallow are of surprising size. Richter found a piece of meat 8 cm. long, from 3 to 4 broad and 2 thick, and Liman found a whole herring rolled up and lodged in the throat. People go to sleep with things in their mouths and these may be aspirated into the air passages. Rubber balls and similar playthings given to children are dangerous and not infrequently cause death. Diehl tells of a man who while springing across a brook aspirated a cigar stump into his air passages and was dead within a few minutes. Articles of food vomited from the stomach may be aspirated into the lungs with fatal results. Accidents of this kind are most likely to happen to children and to drunken adults. When the aspirated particles are small they may be carried into the finest bronchi. Severe hemorrhage or the discharge of an abscess may fill the larynx and cause death by asphyxiation. Cases are reported in which the trachea has been occluded with necrotic tissue from caseous lymph glands that have perforated into the bronchi. In death from accidents of this kind the lungs are found to be inflated.

*Closure of the Nose and Mouth.*—When this is done with the naked hand the force used generally exceeds that necessary and scratches with the nails are frequently left in evidence. When the hand is covered or some soft material is laid over the face and pressure made upon it the marks will, of course, be wanting. Children are often killed in this way or they may be smothered by being covered or by being buried in ashes, loose dirt or in meal or flour. When finely divided material is employed particles may be aspirated into the lungs. Infants and adults in an unconscious state may be suffocated by lying face downward in any soft material. It is said by Okamato, that a few thicknesses of moist Japanese paper placed over the nose and mouth of an infant excludes the air sufficiently to lead to death in a few minutes. Suicide by closing the mouth and nose with the hand is unknown and is probably impossible. Ziemke states that there is recorded one instance of suicide by putting the head under a mass of bed clothing. A woman killed her child and herself in this way. Taylor states that formerly in London a thief would knock his victim senseless and while in this state cover his nose and mouth with a pitch plaster.

*Suffocation by Pressure on the Chest.*—If external pressure on the chest be continued for a short time and be sufficient to markedly retard the respiration death occurs. This is not an uncommon cause of accidental death. For instance, men are digging a trench in a gravel soil and have reached a depth of four to five feet when a mass of earth falls about them. Unless speedily extricated death may result from the external pressure. Indeed, the depth of burial may include no more than the abdomen, and if the pressure be continued it will arrest the movements of the diaphragm and cause death from asphyxia. When people are jammed together or against a wall in a mad rush in the face of some threatening catastrophe some become unconscious and fall on account of the pressure. After falling they are likely to be trampled upon and the injuries resulting from this are generally reckoned as the efficient causes of death. It is easily understood that the mechanism of death by compression is quite different from that induced by any other form of strangulation or suffocation. There is increased blood pressure in every part of the body and ecchymoses are numerous. When the head as well as the trunk is buried two factors may come into play, the partial exclusion of air from the upper passages and the relative immovability of the respiratory muscles. When one is rescued in time to save life the subsequent symptoms will vary with the degree of the pressure and the time it has been borne, but there may be protrusion of the eye balls, fixed pupils, generally in dilatation, possibly complete blindness, and albuminuria. Paralytic symptoms may also be present and psychical disturbances are sometimes observed.

In cases of death the skin has an ashy or bluish-black appearance and on close examination it will be found that this color is not wholly due to cyanosis but that there are numberless ecchymoses.

Death by compression of the thorax is in the great majority of instances accidental. The pressure of the knee on the chest in some cases of homicidal strangulation may be a contributory factor in the causation of death, and Maschka reports a case in which a girl was raped by two men. While one attempted to perform the act the other held her by pressure on her thorax and this killed her.

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—*V. C. V.*

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## *Acidosis*

PERHAPS if the present tendency persists in the elucidation of disease processes we will say that streptococci (or pneumococci), acidosis, and ductless gland disorders cause all diseases. Whether such a trend does or does not persist, there is evidence that there is a great deal of conversational and literary excitement centering about acidosis. Any serious logical discussion of that topic is therefore welcomed, and such is that of Howland and Marriott.[1] Their discussion has particular reference to children, but is not however narrow be-

cause of that reference. They call attention to the fact that acidosis is very loosely applied in medicine and that, as a rule, it is taken to mean that acetone bodies have been found, by qualitative tests, in the urine. They also say that acetone in the urine is not necessarily abnormal. In other words it is not the mere presence of acetone bodies that determines acidosis; acidosis depends upon the relation between acid production and neutralization in the body. The presence of acid in the urine may not be the expression of an acidosis, for a severe acidosis may exist with no abnormal amounts of urinary acid.

There are several ways of discovering the presence of an acidosis. One of these is to discover the ammonia excretion. A high ammonia coefficient always arouses the suspicion of acidosis but unless there is other confirmation, is not conclusive, for it is to be remembered that a high ammonia excretion may result from dietetic causes. Fatal acidosis may occur without any considerable increase of the ammonia coefficient.

Another method is to determine the carbon dioxide tension of the blood. It is diminished in acidosis. It is diminished because there is less alkali (bicarbonate) in the blood. Sodium bicarbonate is the great neutralizer of the body. If there is a relative excess of carbon dioxide over bicarbonate the neutral reaction of the blood moves to the acid side, the respiratory center is stimulated, and by increased pulmonary action, the body attempts to remove the excess of $CO_2$. So, bicarbonate deficiency of the blood is an indication of acidosis, as is also, dyspnœa. The carbonate content may be tested by Sellards' method[2] applied to the serum, or by the alkali tolerance test.[2, 3] The former consists in removing the proteins of blood serum with absolute alcohol and evaporating the filtrate with a few drops of phenolphthalein. Normally a deep purple color appears. According to the degree of carbonate deficiency the color is paler, or absent. The latter test depends upon giving sodium bicarbonate by mouth until the urine is alkaline.

Howland and Marriott have used these methods and give details of cases in which they were applied. They also remark that in absence of proof of presence of acids or loss of bases, a striking evidence of acidosis in children is decrease of urinary secretion.

They make the following statement, "We may lay it down as a general maxim that as hyperpnea indicates acidosis, so hyperpnea indicates alkali therapy, and this for infants or older children." They proceed to say that alkali may be given by any route, but in case rapidity of action is necessary, it is best given intravenously. They mention the method of using the superior longitudinal sinus, or the external jugular or femoral veins. They use only the bicarbonate, —4 per cent for intravenous use; 2 per cent for subcutaneous,—and advise its continuance until the urine is alkaline.

The remarkable things about this contribution are its brevity and lucidity, and the absence of any reference to Fischer's work.

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—*P. G. W.*

## *Recent Studies on Hemophilia*

THERE are few fields in internal medicine which are more obscure than that of the so-called hemorrhagic diseases. The purpuras form a group of poorly differentiated conditions, oftentimes overlapping in their clinical manifestations. Not until we have more exact knowledge of the hemotologic changes will it be possible to classify purpuras satisfactorily or to apply rational treatment.

Hemophilia is one of the hemorrhagic diseases. It has been recognized clinically since 1803, when it was first described by Otto of Philadelphia. He recognized the hereditary nature of the disease and pointed to the fact that it is transmitted by the female, who is herself unaffected by the disease, but may transmit it to her male offspring.

The cause of the tendency to prolonged hemorrhage in hemophilia has been sought by numerous observers. The striking fact has been noted by many that when blood is removed from the hemophilic unmixed with tissue juices, its coagulation time is greatly delayed, often to two or three hours or more. The reason for the delay has been variously given.

According to Howell's theory of coagulation of the blood, there are five essential factors, i.e., prothrombin, antithrombin, thromboplastin, fibrinogen and calcium. With the exception of thromboplastin, which is present in the tissue-juice, all of these substances occur in the circulating blood. Intravascular clotting is prevented by the interaction of the prothrombin and antithrombin. When, however, thromboplastin is added to the blood, it combines with the antithrombin, and thus the prothrombin is set free. The prothrombin is now converted, by the action of calcium, to thrombin. This, in turn, changes the fibrinogen into fibrin, a clot resulting.

With this theory in mind, Addis[1] approached the problem of hemophilia and presented evidence which indicated a qualitative defect in the prothrombin of the blood.

Howell,[2] in 1914, reported his own studies on the blood in hemophilia and other conditions. He devised methods for determining the approximate amounts of antithrombin and of prothrombin in the blood, and applied these methods to the study of three cases of hemophilia, whose spontaneous clotting times varied between two and one-half and five hours, when blood was obtained unmixed with tissue-juice. The importance of careful technic in obtaining the blood was well illustrated by one of his cases. On one occasion the needle missed the vein and only after several trials was it possible to obtain 2 c.c. of blood. When emptied into a tube, this blood clotted in ten minutes, a normal time. With perfect technic, a second specimen was obtained from a vein of the other arm; it required between four and five hours to clot. It is evident, therefore, that admixture of thromboplastin from the tissues greatly lessens the clotting time.

Examination of these bloods for antithrombin content showed a normal amount of this substance, or possibly a slight increase at times, though Howell began his studies with the expectation that a constant excess of antithrombin would be encountered as the cause of the delayed clotting.

The prothrombin content of the blood was also studied. In all the specimens examined from the three patients at various times, there was found a deficiency in the quantity of prothrombin, as compared with normal blood.

Howell, therefore, says of the disease, "Hemophilia may be defined as a condition, limited to the male, in which the coagulation time of the blood is markedly prolonged in consequence of a deficiency in the amount of the contained prothrombin, with the additional characteristic that the defect is transmissible by heredity in accordance with the so-called loss of Nasse." He further says, "The ultimate cause of this condition cannot be stated. So far as we know, the prothrombin present in blood plasma is furnished by the blood platelets, and it is reasonable to assume that the defect in question is referable to some functional change in these elements."

The conclusions of Howell regarding the deficiency in the quantity of prothrombin in the blood in hemophilia have been confirmed by the recent work of Hurwitz and Lucas,[3] who have studied five cases of hemophilia, in all of whom prothrombin deficiency was found, with normal quantities of antithrombin.

By the local application of kephalin, a special phosphatid isolated by Howell (thromboplastic substance), they were able to cause an early arrest of hemorrhage in hemophilics.

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—*R. S. M.*

## *The Pituitary and Growth*

FROM very early times, even those of Galen and Vesalius, it has been suspected that the pituitary had a secretory function, though some intermediate writers believed that this function had only to do with the production of cerebrospinal fluid. Liegevis (1860) was the first to place this gland with the organs of internal secretion—with the ductless glands. But it was not until 1886 that Marie offered evidence that this organ had a sure effect upon the metabolism of the body and that it had an intrinsic importance in the growth processes. Even now, after many years of experimentation and consideration, the mechanism underlying the change in metabolism is not clear. The factor of age is one which must be considered, however, for young animals and adult animals behave differently in their reaction to experimental procedures. If in puppies a part of the gland is removed they become fat, slow, and remain undeveloped physically, and fail to acquire normal secondary sex characteristics. If adult animals are subjected to the same procedure, they lose some, at least, of their acquired sex characteristics and become less fat and less inactive than the younger animals.

During hibernation, the pituitary undergoes a sort of physiologic atrophy and this process is associated with increase of fat, lethargy, low temperature, and general physical inactivity, symptoms which are similar to those resulting from experimental damage to the gland. As the period of hibernation passes, the pituitary becomes normal in appearance and the symptoms are lost. Feeding gland substance after destruction of the gland produces similar speeding up of metabolism.

Not a few experiments upon the effects of feeding pituitary substance to, or injecting pituitary extracts into, animals have been made—with very contradictory results. Crowe, Cushing and Homans,[1] for instance, found that repeated subcutaneous injections of sterile extracts or emulsions of the whole gland, or of posterior lobe alone, given subcutaneously, were apt to lead to emaciation. Caselli, on the other hand, was able to observe no appreciable effect upon growth after long continued injections of glycerine extracts of the whole gland. But Cushing nevertheless believed that growth was retarded by feeding pituitary material. Sandri reported negative results after feeding young mice with bovine anterior lobe, but feeding with posterior lobe arrested development. Schaefer on the other hand, observed a definite increase in growth of young rats fed upon anterior lobe extracts. Aldrich and Lewis and Miller[2] could perceive no obvious effects produced by feeding pituitary substance to young rats. Wulsen[3] using fresh anterior lobe of ox pituitary observed retardation of growth. Behrenroth is, Goetsch says, the only author who makes special mention of an accompanying effect upon another of the ductless glands. He reported that pituitrin given at proper intervals produced premature development of the sexual organs and coincident increased sexual activity on the part of the animals (young rats).

It was because of the contradictory results of pituitary treatment as reported in the literature, and because of the quite obvious correlation of the sexual glands with the pituitary, that Goetsch has carried out a series of experiments[4] with young rats, of known pedigree, kept under the best conditions, fed with pituitary substances in the form of powdered extracts of the various parts of the gland. His reported results are as follows:

Dried powdered pituitary extract derived from both anterior and posterior lobes, when fed to young rats in excessive doses (0.1 gm. daily), causes failure to gain in weight, loss of appetite, increased peristalsis, a mild enteritis, and certain nervous manifestations, such as muscular tremors and weakness of the hind limbs. The latter symptoms seem to be due to posterior lobe elements in the extract. When whole gland is fed over a short period of time, not in excessive doses, it causes a more rapid growth and development, gain in weight, larger nipples in the female, and a coarser, drier, harsher growth of hair than are seen in either control animals or after similar administration of ovarian extract in equivalent dosage. Whole gland extracts also influence the female sex glands, increasing their development and activity. In males there is an analogous stimulation. In other words, sexual precocity results in either sex.

Feeding with anterior lobe extracts produces the same effects as feeding whole gland, and also exerts a stimulant effect upon the fetuses *in utero*. Extracts of posterior lobe on the contrary seem to have a retarding influence upon the sex glands, and one which is quite similar to the effect of ovarian extract on the testes. Moreover posterior lobe extracts do not stimulate growth in general. Large doses cause loss of weight.

Ovarian extracts have stimulating effects upon the female and retarding influence upon the male sexual development.

These results explain, as Goetsch says, the fact that in acromegaly which is due to overfunction of the anterior lobe, the early stages are characterized by

increased sexual activity and in the late stages loss of sexual function. In the later stages the pituitary is exhausted or destroyed.

The chief value of the work seems to be that it offers indications that pituitary therapy may be successful. Goetsch points to the fact that pituitary feeding in loss of sexual activity due to hypophysis disease has resulted in a return, partial or complete, of those activities.[5] He also suggests supplementary feeding after surgical operations upon the pituitary, and also in conditions characterized by genital aplasia, adiposity, and under-development, pituitary feeding may be valuable, with extracts of other glands when necessary. Also it is possible to treat certain conditions of sexual overactivity with posterior lobe extracts, or, in males, with corpus luteum extracts.

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—*P. G. W.*

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## *Splenectomy*

IN a recent number of this Journal,[1] attention was called to the relation between the liver and spleen, and to the function of the spleen. In the remarks made at that time it was stated that "heretofore splenectomy has been an experiment. It has been done as a last resort. But if in certain anemic conditions it can be shown that abnormal hemolysis is fundamental, and that the anemia is due to it rather than to a lack of production of blood cells or pigment, then it will be logical and not merely empirical to remove the organ which furnishes the normal augmenter of hemolysis." At the same time it may be that experimental removal of the spleen may show whether or not the spleen is overfunctioning in producing the augmenting substance which activates, so to speak, the normal hemolytic substances of the liver.

In a recent article, W. J. Mayo[2] recounts the results he has had with splenectomy. He divides the cases into four groups; those in which the spleen is the seat of a primary tumor; those in which the spleen is the seat of an infectious process; those in which it shows enlargement associated with disease of the liver; and those which are associated with the so-called blood diseases. The first group he leaves out of account.

Among cases belonging to the second group, he places one of, apparently, primary tuberculosis. Temporary improvement was followed by death after several months from general miliary tuberculosis. Also belonging in this group, were two cases of chronic syphilis with splenomegaly and anemia. After removal of the spleen there was a "marvelous" improvement of the anemia. In other conditions such as chronic recurrent sepsis, the spleen has been removed in seven instances. The outcome is not stated. The context indicates that it was not good.

The results in the third group have been not much better. In one case of

Hanot's cirrhosis, splenectomy gave undoubted benefit. In four instances of portal cirrhosis, splenectomy has been performed with excellent temporary results—disappearance of the ascites and anemia. If, as is suggested, the noxæ which cause cirrhosis come from the intestinal tract then there seems to be no good reason for making the experiment, unless the experiment is justified by a purely temporary relief. We believe such an experiment is justified, but are unable from the data to expect a cure.

In the last group the results of splenectomy are truly brilliant. In splenic anemia, Banti's disease, and Gaucher's disease, cases were definitely improved, and, if operated upon early in the disease, may be cured. Most interesting perhaps in this group is hemolytic jaundice. In this group splenectomy has been done in nine cases with striking results. "Within twenty-four hours the jaundice begins to disappear and in a few days the patients, perhaps for the first time, have clear complexions; the anemia is rapidly overcome and they remain well." In pernicious anemia also, after an experience with 19 patients, the results seemed promising in selected and early cases.

These surgical experiences bear out the conclusions which are drawn from experimental work. The brilliant results appear in cases in which hemolysis is the salient feature, and in such cases it is the removal of the source of hemolysis augmenting substance which is productive of good. The results must also always be good in cases of primary diseases of the spleen which tend to spread therefrom, or which will tend to render the organ useless. It is possible that in some cases of pernicious anemia, part of the fault lies in splenic overactivity; in such cases splenectomy should be valuable. In other cases, those of purely myeloid origin, it would seem to be useless to remove this organ. In any case of anemia, a temporary improvement might be expected, if it be true that the spleen has a part to play in normal blood destruction.

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—*P. G. W.*